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UTILITY PATENT APPLICATION TRANSMITTAL
(Small Entity)

(Only for new nonprovisional applications under 37 CFR 1.53(b))

Docket No. 004.00191
Total Pages in this Submission

TO THE ASSISTANT COMMISSIONER FOR PATENTS
Box Patent Application
Washington, D.C. 20231

Transmitted herewith for filing under 35 U.S.C. 111(a) and 37 C.F.R. 1.53(b) is a new utility patent application for an invention entitled:

T-TYPE CALCIUM CHANNEL

and invented by:

Ming Li

If a CONTINUATION APPLICATION, *check appropriate box and supply the requisite information:*

☐ **Continuation** ☐ **Divisional** ☐ **Continuation-in-part (CIP)** of prior application No.: _____

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Enclosed are:

Application Elements

1. ☐ Filing fee as calculated and transmitted as described below
2. ☒ Specification having **86** pages and including the following:
   - a. ☒ Descriptive Title of the Invention
   - b. ☒ Cross References to Related Applications *(if applicable)*
   - c. ☒ Statement Regarding Federally-sponsored Research/Development *(if applicable)*
   - d. ☐ Reference to Microfiche Appendix *(if applicable)*
   - e. ☒ Background of the Invention
   - f. ☒ Brief Summary of the Invention
   - g. ☒ Brief Description of the Drawings *(if drawings filed)*
   - h. ☒ Detailed Description
   - i. ☒ Claim(s) as Classified Below
   - j. ☒ Abstract of the Disclosure

UTILITY PATENT APPLICATION TRANSMITTAL
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Application Elements (Continued)

3. ☒ Drawing(s) *(when necessary as prescribed by 35 USC 113)*
   a. ☒ Formal b. ☐ Informal Number of Sheets 15

4. ☐ Oath or Declaration
   a. ☐ Newly executed *(original or copy)* ☐ Unexecuted
   b. ☐ Copy from a prior application (37 CFR 1.63(d)) *(for continuation/divisional application only)*
   c. ☐ With Power of Attorney ☐ Without Power of Attorney
   d. ☐ *DELETION OF INVENTOR(S)*
   Signed statement attached deleting inventor(s) named in the prior application, see 37 C.F.R. 1.63(d)(2) and 1.33(b).

5. ☐ Incorporation By Reference *(usable if Box 4b is checked)*
The entire disclosure of the prior application, from which a copy of the oath or declaration is supplied under Box 4b, is considered as being part of the disclosure of the accompanying application and is hereby incorporated by reference therein.

6. ☐ Computer Program in Microfiche

7. ☒ Genetic Sequence Submission *(if applicable, all must be included)*
   a. ☒ Paper Copy
   b. ☐ Computer Readable Copy
   c. ☐ Statement Verifying Identical Paper and Computer Readable Copy

Accompanying Application Parts

8. ☐ Assignment Papers *(cover sheet & documents)*

9. ☐ 37 CFR 3.73(b) Statement *(when there is an assignee)*

10. ☐ English Translation Document *(if applicable)*

11. ☐ Information Disclosure Statement/PTO-1449 ☐ Copies of IDS Citations

12. ☐ Preliminary Amendment

13. ☒ Acknowledgment postcard

14. ☒ Certificate of Mailing
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UTILITY PATENT APPLICATION TRANSMITTAL
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Accompanying Application Parts (Continued)

15. ☐ Certified Copy of Priority Document(s) *(if foreign priority is claimed)*

16. ☐ Small Entity Statement(s) - Specify Number of Statements Submitted: _____

17. ☐ Additional Enclosures *(please identify below):*

Fee Calculation and Transmittal

CLAIMS AS FILED

For	#Filed	#Allowed	#Extra		Rate	Fee
Total Claims	60	- 20 =	40	x	$9.00	$360.00
Indep. Claims	13	- 3 =	10	x	$39.00	$390.00
Multiple Dependent Claims (check if applicable) ☐						$0.00
					BASIC FEE	$380.00
OTHER FEE *(specify purpose)* _____						$0.00
					TOTAL FILING FEE	$1,130.00

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- ☐ Charge any additional filing fees required under 37 C.F.R. 1.16 and 1.17.
- ☐ Charge the issue fee set in 37 C.F.R. 1.18 at the mailing of the Notice of Allowance, pursuant to 37 C.F.R. 1.311(b).

Dated: **August 26, 1999**

Susan J. Braman

Signature

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CERTIFICATE OF MAILING BY "EXPRESS MAIL" (37 CFR 1.10) Applicant(s): **Ming Li**	Docket No. **004.00191**

Serial No.	Filing Date	Examiner	Group Art Unit
To Be Assigned	**Herewith**	**Unknown**	**Unknown**

Invention:

T-TYPE CALCIUM CHANNEL

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Invention:

T-TYPE CALCIUM CHANNEL

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Serial No. **To Be Assigned**	Filing Date **Herewith**	Examiner **Unknown**	Group Art Unit **Unknown**

Invention:

T-TYPE CALCIUM CHANNEL

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TITLE: T-TYPE CALCIUM CHANNEL

INVENTOR: Ming Li

DOCKET NO.:004.00191

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T-TYPE CALCIUM CHANNEL

This application claims priority of U.S. Provisional Patent Application No. 60/098,004, filed August 26, 1998, and of U.S. Provisional Patent Application No. 60/117,399, filed January 27, 1999.

The subject matter of this application was made with support from the United States Government under National Institutes of Health Grant No. 5-20174. The U.S. Government may have certain rights in this invention.

FIELD OF THE INVENTION

The present invention relates generally to calcium channel proteins, and more particularly to pancreatic T-type calcium channel proteins and uses thereof.

BACKGROUND OF THE INVENTION

Throughout this application various publications are referenced, many in parenthesis. Full citations for each of these publications are provided at the end of the Detailed Description. The disclosures of each of these publications in their entireties are hereby incorporated by reference in this application.

Insulin secretion from pancreatic β-cells is the primary physiological mechanism of blood glucose regulation. A rise in blood glucose concentration stimulates release of insulin from the pancreas, which in turn promotes glucose uptake in peripheral tissues and consequently lowers blood glucose levels, reestablishing euglycemia. Non-insulin dependent diabetes mellitus (NIDDM)(type II diabetes) is associated with an impairment in glucose-induced insulin secretion in pancreatic β-cells (Vague and Moulin, 1982).

Voltage-gated Ca^{2+} channels mediate a rapidly activated inward movement of Ca^{2+} ions that underlies the

stimulation of insulin secretion in β-cells (Boyd III 1991). In different tissues, four types of Ca^{2+} channels have been described (L(P/Q), T, N, and E channels). The purified L-type Ca^{2+} channel consists of five subunits: α_1, α_2, β, γ, δ (Catterall 1991). The primary structure of the α_1 subunit is organized in four homologous domains containing six transmembrane segments (Catterall 1988).

Rat and human pancreatic β-cells are equipped with L-type and T-type Ca^{2+} channels (Hiriart and Matteson, 1988; Davalli et al., 1996). L-type Ca^{2+} channels, activated at high voltages and having large unitary conductance and dihydropyridine-sensitivity, are considered the major pipeline for Ca^{2+} influx into the β-cell (Keahey et al., 1989). In contrast, T-type calcium channels activate at low voltages and have small unitary conductance and dihydropyridine-insensitivity.

The physiological function of T-type Ca^{2+} channels in β-cell insulin-secretion has been demonstrated (Bhattacharjee et al., 1997). These channels facilitate exocytosis by enhancing electrical activity in these cells. L-type and T-type Ca^{2+} channels, under normal conditions, work in concert promoting the rise in $[Ca^{2+}]_i$ during glucose-stimulated insulin secretion. In β-cells, over-expressed T-type Ca^{2+} channels may be, at least in part, responsible for the hyper-responsiveness of insulin secretion to non-glucose depolarizing stimuli in GK rat and in rat with NIDDM induced by neonatal injection of streptozotocin (Kato et al., 1994; Kato et al., 1996). However, over-expressed T-type calcium channels over time will ultimately lead to an elevation of basal Ca^{2+} through it's window current properties. Therefore, there is a dual effect of T-type Ca^{2+} channels in β-cells depending upon channel number and membrane potential.

Two isoforms of L-type Ca^{2+} channel $\alpha 1$ subunits have been identified in β-cells (Seino et al., 1992; Yaney et al., 1992). The rat neuronal T-type calcium channel has recently been cloned (Perez-Reyes et al., 1998). Other subunits of T-type Ca^{2+} channel have yet to be identified.

Given the evidence that T-type calcium channels are associated with type II diabetes, a need exists to further characterize T-type calcium channels.

SUMMARY OF THE INVENTION

To this end, the subject invention provides an isolated nucleic acid molecule encoding a pancreatic T-type calcium channel. The invention also provides an antisense nucleic acid molecule complementary to at least a portion of the mRNA encoding the pancreatic T-type calcium channel.

The isolated nucleic acid molecules of the invention can be inserted into suitable expression vectors and/or host cells. Expression of the nucleic acid molecules encoding the pancreatic T-type calcium channel results in production of pancreatic T-type calcium channel in a host cell. Expression of the antisense nucleic acid molecules in a host cell results in decreased expression of the pancreatic T-type calcium channel.

The invention further provides a ribozyme having a recognition sequence complementary to a portion of mRNA encoding a pancreatic T-type calcium channel. The ribozyme can be introduced into a cell to also achieve decreased expression of pancreatic T-type calcium channel in the cell.

The invention further provides a method of screening a substance for the ability of the substance to modify T-type calcium channel function, and a method of obtaining DNA encoding a pancreatic T-type calcium channel.

Further provided is an isolated nucleic acid molecule encoding a pancreatic T-type calcium channel, wherein the nucleic acid molecule encodes a first amino acid sequence having at least 90% amino acid identity to a second amino acid sequence. The second amino acid sequence is as shown in SEQ ID NO:2.

The invention further provides a DNA oligomer capable of hybridizing to a nucleic acid molecule encoding a pancreatic T-type calcium channel. The DNA oligomer can be used in a method of detecting presence of a pancreatic T-type calcium channel in a sample, which method is also provided by the subject invention.

The invention also provides an isolated pancreatic T-type calcium channel protein, and antibodies or antibody fragments specific for the pancreatic T-type calcium channel protein. The antibodies and antibody fragments can be used to detect the presence of the pancreatic T-type calcium channel protein in samples. Further provided is an isolated pancreatic T-type calcium channel protein encoded by a first amino acid sequence having at least 90% amino acid identity to a second amino acid sequence, the second amino acid sequence as shown in SEQ ID NO:2.

The subject invention further provides a method of modifying insulin secretion by pancreatic beta cells, the method comprising modifying levels of functional T type calcium channels in the pancreatic beta cells. The invention further provides a method of treating type II diabetes in a subject, the method comprising administering to the subject an amount of a compound effective to modify levels of functional T type calcium channel in the pancreatic beta cells of the subject.

The invention also provides a method of modifying basal calcium levels in cells, a method of modifying the

action potential of L type calcium channels in cells, a method of modifying pancreatic beta cell death, a method of modifying pancreatic beta cell proliferation, and a method of modifying calcium influx through L type calcium channels in cells, each of the methods comprising modifying levels of functional T type calcium channels in the cells.

BRIEF DESCRIPTION OF THE DRAWINGS

These and other features and advantages of this invention will be evident from the following detailed description of preferred embodiments when read in conjunction with the accompanying drawings in which:

Fig. 1A illustrates a comparison of the nucleotide sequence of α_1G-INS (1) and α_1G (2) at the 5'-end regions (aa1-67 of α_1G). The four insertions are indicated with arrow heads. The capital ATG represents the start codon for each cDNA;

Fig. 1B is a schematic illustration representing partial rat genomic nucleotide composition between Domain III and IV. Genomic DNA contained an exon specific to α_1G (shaded circle) and an exon specific to the α_1 subunit of T-type Ca^{2+} deduced from INS-1 (shaded rectangle) between 4845 and 5256 of the cDNA sequence. Other exons (open rectangles) are identical between the two cDNAs. The bold letters indicate the nucleotides coding Gly-1667;

Figs. 2A-2D illustrate expression of α_1G-INS in Xenopus oocytes. Fig. 2A illustrates 40 mM Ca^{2+} currents elicited by depolarizing pulses from - 60 to 40 mV. Fig. 2B illustrates time constants of activation and inactivation measured at test potentials between -30 and 30 mV. The time constants of activation were obtained by fitting the increasing portion (activation) of currents with the Hodgkin-Huxley equation where the m value was

designated as four (n = 6). The time constants of inactivation were obtained by single exponential fitting (n = 6). Fig. 2C illustrates voltage-dependent conductance (n = 7) and Fig. 2D illustrates steady-state inactivation (n = 3) of expressed currents in oocytes. The holding potential for Figs. 2C and 2D was -80 mV. The currents in Fig. 2D were measured at -10 mV after varying 1000 ms pre-pulse potentials. Peak currents were normalized to the maximum current and then averaged (error bars represent SE);

Figs. 3A and 3B illustrate accumulative dose response relationships of the inhibitory effects of mibefradil on T- and L-type Ca^{2+} currents. Currents were measured with the whole-cell patch clamp configuration. Data from four experiments were normalized individually and than plotted as mean ± standard error. Fig. 3A illustrates curve which was generated by fitting the data using one-to-one binding curve according to the equation $1/(1 + [\text{mibefradil}]/K_d)$. Fig. 3B is a dose response of L-type Ca^{2+} current obtained when perfusion of solutions containing different concentrations of mibefradil;

Fig. 4 illustrates reversibility of the inhibition of T and L-type currents by $NiCl_2$ and mibefradil, respectively. Open and solid circles represent the T-type Ca^{2+} current recorded before and after $NiCl_2$ (2 μl of 30 μM) and mibefradil (2 μl of 10 μM) were administrated, respectively. The open squares represent the L-type Ca^{2+} current recorded before and after mibefradil (2 μl of 10 μM) was administrated with perforated patch clamp configuration. The T-type Ca^{2+} current was measured at -30 mV with a holding potential of -80 mV with whole cell configuration. Arrow indicates the time when the drugs were delivered. n =3 for each group experiments;

Figs. 5A and 5B illustrate the long-term effect of mibefradil (10 nM) on L- and T- Ca^{2+} currents in the perforated-patch configuration. In Fig. 5A, solid and open circles represent the L-type Ca^{2+} current recorded in the cells with and without administration of mibefradil, respectively. Solid triangles represent T-type Ca^{2+} currents recorded in the cells after administrating mibefradil. Mibefradil were delivered at time zero. n = 4 for each group experiments. In Fig. 5B, cells were cultured in medium with or without co-incubating 10 nM mibefradil for 2 hours. The current densities were recorded with perforated patch clamp configuration. n = 14 for each group experiments;

Fig. 6A illustrates accumulation of dm-mibefradil in the cells measured with mass spectrometry. The cells were first incubated with mibefradil (20 μM) for the duration indicated on the figure (n = 3). The inset (Fig. 6B) shows the primary data of mass spectrometry indicating peaks at 496 and 424, which correspond to mibefradil and dm-mibefradil, respectively;

Fig. 7A illustrates the effect of mibefradil and dm-mibefradil on L-type Ca^{2+} currents from inside cells. n = 8, *, $p < 0.01$ to the control;

Fig. 7B illustrates the effect of mibefradil or dm-mibefradil on T-type Ca^{2+} current from inside cells n = 4. All data were collected at 5 min after formation of whole cell patch. The pipette solution contained 1 μM of drug;

Fig. 8 illustrates basal $[Ca^{2+}]_i$ measured in an INS-1 cell. T-type calcium channel antagonist mibefradil (1 μM) reduced basal $[Ca^{2+}]_i$ in a single cell in the bath solution without glucose. The $[Ca^{2+}]_i$ was measured with the emission ratio of Fura-2 AM (F380/F340) then

calibrated with the standard solution purchased from Molecular Probes Inc. (OR);

Fig. 9A illustrates that intracellular perfusion of a solution containing 272 nM free calcium concentration inhibits the L-type calcium current. Currents were elicited by a step voltage to +10 mV, with holding potential of -80 mV;

Fig. 9B illustrates the effect of perfusing in high calcium concentration on the IV calcium current relationship. Closed circles represent the cell before perfusion, and open circles represent perfusion of 272 nM free calcium;

Fig. 9C illustrates the effect of intracellular perfusion of different calcium concentrations on L-type calcium current over time. Squares represent perfusion from high calcium to low calcium (intracellular solution contained 632 nM then perfused by a solution with 10 mM EGTA), triangles represent perfusion from low calcium to 272 nM calcium, and circles represent low calcium to 632 nM calcium;

Fig. 9D illustrates the effect of high calcium on the T-type calcium channel current. Tail currents were elicited by a voltage step to -30 mV for 10 ms;

Fig. 10 illustrates that reestablishment of basal calcium causes stereotyped calcium influx. A cell was twice perfused with 50 mM KCl with an intervening perfusion of the original bath solution to restore membrane potential;

Fig. 11 illustrates that elevated basal Ca^{2+} causes a defect in the Ca^{2+} transient. A cell was twice perfused with 50 mM KCl with an intervening perfusion of the original bath solution to restore membrane potential. The second perfusion occurred prior to reestablishment of the original basal $[Ca^{2+}]_i$ of about 60 nM;

Fig. 12 illustrates a model for glucose-stimulated insulin release;

Fig. 13 illustrates that mibefradil (1 μM) blocks T- and L-type Ca^{2+} current in INS-1 cells. The relative current of T type Ca channel is obtained by measuring their slow deactivated tail current (n = 8);

Fig. 14 illustrates that mibefradil and $NiCl_2$ reversibly block T type Ca^{2+} current in INS-1 cells. Drugs were administered into the recording chamber at 180 seconds from the beginning of recording. N = 3;

Fig. 15 illustrates the activation and inactivation curves for INS-1 cells, revealing a "window current";

Fig. 16 illustrates the effect of $NiCl_2$, mibefradil, and nifedipine on basal insulin secretion in NIT-1 cells. The glucose concentration is 3 mM in the experiments;

Fig. 17 illustrates that the T type calcium channel antagonist $NiCl_2$ (30 μM) reduced the frequency of transient spontaneous elevation of $[Ca^{2+}]_i$, in a single cell in the bath solution without glucose;

Fig. 18 illustrates the effect of 30 mM $NiCl_2$ on the $[Ca^{2+}]_i$ under non-stimulus conditions. Data was collected from the cells with "high" initial basal $[Ca^{2+}]_i$ (about 100 nM). n = 13;

Figs. 19A and 19B illustrate that hyperpolarization induced an increase in number of action potentials and a decrease in onset latencies. N = 40;

Figs. 20A and 20B illustrate the dose-dependent effect of $NiCl_2$ on insulin secretion. Cells were placed in a medium containing 11.1 mM glucose and a decrease in onset latencies. N = 40;

Fig. 21 illustrates "run-up" in whole cell recording;

Fig. 22 illustrates KCl induced Ca^{2+} influx in the INS-1 cells treated with streptozotocin. n = 13;

Fig. 23A-23F illustrate the results of cytokine treatment. LVA Ca^{2+} currents were induced by cytokine treatment (IL-1β, 25 U/ml; IFNγ, 300 U/ml) for 6 h in primary cultured mouse islet cells, but not in α-TC1 cells. An LVA current was elicited by a -40 mV test pulse in an islet cell (Fig. 23A), but the same current was not detected in α-TC1 cells (Fig. 23C). The Ca^{2+} current density-voltage relationships obtained from islet cells (Fig. 23B) and α-TC1 cells (Fig. 23D) with and without cytokine treatment are shown. The open circles represent the current densities of untreated cells (n = 10 for islet cells; n = 20 for α-TC1 cells), and the filled circles represent the current densities of cells treated by cytokines (n = 21 for islet cells; n = 21 for α-TC1 cells). The recordings were elicited by voltages ranging from -50 to +20 mV for 100 msec. All experiments were performed at -80 mV. Fig. 23E shows steady state inactivation of LVA tail currents elicited by a 10-msec depolarizing (-10 mV) pulse followed by a 50-msec hyperpolarizing pulse (-100 mV), with a holding potential of -80 mV. Fig. 23F shows that $NiCl_2$ (10 μM) blocked the cytokine induced LVA Ca^{2+} current elicited at a -30 mV step pulse in an islet cell;

Figs. 24A and 24B illustrate the effects of cytokines on $[Ca^{2+}]_i$ in mouse islet cells and α-TC1 cells. In Fig. 24A, basal $[Ca^{2+}]_i$ of primary cultured mouse islet cells was approximately 3-fold higher after cytokine treatment. $NiCl_2$ (10 μM), but not nifedipine (10 μM), prevented the increase in $[Ca^{2+}]_i$. In Fig. 24B, basal $[Ca^{2+}]_i$ in α-TC1 cells was unaffected by cytokine treatment. Cytokine treatment consisted of IL-1β (25 U/ml) and IFNγ (300 U/ml) for 6 h; and

Figs. 25A and 25B illustrate the effects of $NiCl_2$ on cytokine-induced β-TC3 cell death. $NiCl_2$ (20 μM)

significantly reduced cell death induced by cytokines in both a time (Fig. 25A) and dose-dependent (Fig. 25B) manner (n = 3). Cytokine treatment consisted of IL-1β (25 U/ml), IFNγ (100 U/ml), and TNFα (100 U/ml) in Fig. 25A and of IL-1β (25 U/ml), TNFα (100 U/ml), and various concentrations of IFNγ as indicated in Fig. 25A. The first dose, 0, represents zero concentration for all three cytokines. The concentration of nifedipine was 10 μM in both Fig. 25A and Fig. 25B.

DETAILED DESCRIPTION OF THE INVENTION

The term "nucleic acid", as used herein, refers to either DNA or RNA. "Nucleic acid sequence" or "polynucleotide sequence" refers to a single- or double-stranded polymer of deoxyribonucleotide or ribonucleotide bases read from the 5' to the 3' end. It includes both self-replicating plasmids, infectious polymers of DNA or RNA, and nonfunctional DNA or RNA.

"Isolated" nucleic acid refers to nucleic acid which has been separated from an organism in a substantially purified form (i.e. substantially free of other substances originating from that organism), and to synthetic nucleic acid.

By a nucleic acid sequence "homologous to" or "complementary to", it is meant a nucleic acid that selectively hybridizes, duplexes or binds to DNA sequences encoding the protein (channel) or portions thereof when the DNA sequences encoding the protein are present in a human genomic or cDNA library. A DNA sequence which is similar or complementary to a target sequence can include sequences which are shorter or longer than the target sequence so long as they meet the functional test set forth.

Typically, the hybridization is done in a Southern blot protocol using a 0.2X SSC, 0.1% SDS, 65°C wash. The term "SSC" refers to a citrate-saline solution of 0.15M sodium chloride and 20 mM sodium citrate. Solutions are often expressed as multiples or fractions of this concentration. For example, 6X SSC refers to a solution having a sodium chloride and sodium citrate concentration of 6 times this amount or 0.9 M sodium chloride and 120 mM sodium citrate. 0.2X SSC refers to a solution 0.2 times the SSC concentration or 0.03M sodium chloride and 4 mM sodium citrate.

The phrase "nucleic acid molecule encoding" refers to a nucleic acid molecule which directs the expression of a specific protein or peptide. The nucleic acid sequences include both the DNA strand sequence that is transcribed into RNA and the RNA sequence that is translated into protein or peptide. The nucleic acid molecule includes both the full length nucleic acid sequences as well as non-full length sequences derived from the full length protein. It being further understood that the sequence includes the degenerate codons of the native sequence or sequences which may be introduced to provide codon preference in a specific host cell.

The term "located upstream" as used herein refers to linkage of a promoter upstream from a nucleic acid (DNA) sequence such that the promoter mediates transcription of the nucleic acid (DNA) sequence.

The term "vector", refers to viral expression systems, autonomous self-replicating circular DNA (plasmids), and includes both expression and nonexpression plasmids. Where a recombinant microorganism or cell is described as hosting an "expression vector," this includes both extrachromosomal

circular DNA and DNA that has been incorporated into the host chromosome(s). Where a vector is being maintained by a host cell, the vector may either be stably replicated by the cells during mitosis as an autonomous structure, or the vector may be incorporated within the host's genome.

The term "plasmid" refers to an autonomous circular DNA molecule capable of replication in a cell, and includes both the expression and nonexpression types. Where a recombinant microorganism or cell is described as hosting an "expression plasmid", this includes latent viral DNA integrated into the host chromosome(s). Where a plasmid is being maintained by a host cell, the plasmid is either being stably replicated by the cell during mitosis as an autonomous structure, or the plasmid is incorporated within the host's genome.

The phrase "heterologous protein" or "recombinantly produced heterologous protein" refers to a peptide or protein of interest produced using cells that do not have an endogenous copy of DNA able to express the peptide or protein of interest. The cells produce the peptide or protein because they have been genetically altered by the introduction of the appropriate nucleic acid sequences. The recombinant peptide or protein will not be found in association with peptides or proteins and other subcellular components normally associated with the cells producing the peptide or protein.

The following terms are used to describe the sequence relationships between two or more nucleic acid molecules or polynucleotides, or between two or more amino acid sequences of peptides or proteins: "reference sequence", "comparison window", "sequence identity", "sequence homology", "percentage of sequence identity", "percentage of sequence homology", "substantial

identity", and "substantial homology". A "reference sequence" is a defined sequence used as a basis for a sequence comparison; a reference sequence may be a subset of a larger sequence, for example, as a segment of a full-length cDNA or gene sequence given in a sequence listing or may comprise a complete cDNA or gene sequence.

Optimal alignment of sequences for aligning a comparison window may be conducted, for example, by the local homology algorithm of Smith and Waterman (1981), by the homology alignment algorithm of Needleman and Wunsch (1970), by the search for similarity method of Pearson and Lipman (1988), or by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package Release 7.0, Genetics Computer Group, 575 Science Dr., Madison, Wis.).

As applied to nucleic acid molecules or polynucleotides, the terms "substantial identity" or "substantial sequence identity" mean that two nucleic acid sequences, when optimally aligned (see above), share at least 90 percent sequence identity, preferably at least 95 percent sequence identity, more preferably at least 96, 97, 98 or 99 percent sequence identity.

"Percentage nucleotide (or nucleic acid) identity" or "percentage nucleotide (or nucleic acid) sequence identity" refers to a comparison of the nucleotides of two nucleic acid molecules which, when optimally aligned, have approximately the designated percentage of the same nucleotides. For example, "95% nucleotide identity" refers to a comparison of the nucleotides of two nucleic acid molecules which when optimally aligned have 95% nucleotide identity. Preferably, nucleotide positions which are not identical differ by redundant nucleotide substitutions (the nucleotide substitution does not change the amino acid encoded by the particular codon).

As further applied to nucleic acid molecules or polynucleotides, the terms "substantial homology" or "substantial sequence homology" mean that two nucleic acid sequences, when optimally aligned (see above), share at least 90 percent sequence homology, preferably at least 95 percent sequence homology, more preferably at least 96, 97, 98 or 99 percent sequence homology.

"Percentage nucleotide (or nucleic acid) homology" or "percentage nucleotide (or nucleic acid) sequence homology" refers to a comparison of the nucleotides of two nucleic acid molecules which, when optimally aligned, have approximately the designated percentage of the same nucleotides or nucleotides which are not identical but differ by redundant nucleotide substitutions (the nucleotide substitution does not change the amino acid encoded by the particular codon). For example, "95% nucleotide homology" refers to a comparison of the nucleotides of two nucleic acid molecules which when optimally aligned have 95% nucleotide homology.

As applied to polypeptides, the terms "substantial identity" or "substantial sequence identity" mean that two peptide sequences, when optimally aligned, such as by the programs GAP or BESTFIT using default gap, share at least 90 percent sequence identity, preferably at least 95 percent sequence identity, more preferably at least 96, 97, 98 or 99 percent sequence identity.

"Percentage amino acid identity" or "percentage amino acid sequence identity" refers to a comparison of the amino acids of two polypeptides which, when optimally aligned, have approximately the designated percentage of the same amino acids. For example, "95% amino acid identity" refers to a comparison of the amino acids of two polypeptides which when optimally aligned have 95% amino acid identity. Preferably, residue positions which

are not identical differ by conservative amino acid substitutions. For example, the substitution of amino acids having similar chemical properties such as charge or polarity are not likely to affect the properties of a protein. Examples include glutamine for asparagine or glutamic acid for aspartic acid.

As further applied to polypeptides, the terms "substantial homology" or "substantial sequence homology" mean that two peptide sequences, when optimally aligned, such as by the programs GAP or BESTFIT using default gap, share at least 90 percent sequence homology, preferably at least 95 percent sequence homology, more preferably at least 96, 97, 98 or 99 percent sequence homology.

"Percentage amino acid homology" or "percentage amino acid sequence homology" refers to a comparison of the amino acids of two polypeptides which, when optimally aligned, have approximately the designated percentage of the same amino acids or conservatively substituted amino acids. For example, "95% amino acid homology" refers to a comparison of the amino acids of two polypeptides which when optimally aligned have 95% amino acid homology. As used herein, homology refers to identical amino acids or residue positions which are not identical but differ only by conservative amino acid substitutions. For example, the substitution of amino acids having similar chemical properties such as charge or polarity are not likely to affect the properties of a protein. Examples include glutamine for asparagine or glutamic acid for aspartic acid.

The phrase "substantially purified" or "isolated" when referring to a protein (or peptide), means a chemical composition which is essentially free of other cellular components. It is preferably in a homogeneous state although it can be in either a dry or aqueous

solution. Purity and homogeneity are typically determined using analytical chemistry techniques such as polyacrylamide gel electrophoresis or high performance liquid chromatography. A protein (or peptide) which is the predominant species present in a preparation is substantially purified. Generally, a substantially purified or isolated protein (or peptide) will comprise more than 80% of all macromolecular species present in the preparation. Preferably, the protein (or peptide) is purified to represent greater than 90% of all macromolecular species present. More preferably the protein (or peptide) is purified to greater than 95%, and most preferably the protein (or peptide) is purified to essential homogeneity, wherein other macromolecular species are not detected by conventional techniques. A "substantially purified" or "isolated" protein (or peptide) can be separated from an organism, synthetically or chemically produced, or recombinantly produced.

"Biological sample" or "sample" as used herein refers to any sample obtained from a living organism or from an organism that has died. Examples of biological samples include body fluids and tissue specimens.

High stringent hybridization conditions are selected at about 5°C lower than the thermal melting point (Tm) for the specific sequence at a defined ionic strength and pH. The Tm is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe. Typically, stringent conditions will be those in which the salt concentration is at least about 0.02 molar at pH 7 and the temperature is at least about 60°C. As other factors may significantly affect the stringency of hybridization, including, among others, base composition and size of the complementary strands, the presence of organic solvents,

i.e. salt or formamide concentration, and the extent of base mismatching, the combination of parameters is more important than the absolute measure of any one. High stringency may be attained, for example, by overnight hybridization at about 68°C in a 6X SSC solution, washing at room temperature with 6X SSC solution, followed by washing at about 68°C in a 6X SSC solution then in a 0.6X SSX solution.

Hybridization with moderate stringency may be attained, for example, by: 1) filter pre-hybridizing and hybridizing with a solution of 3X sodium chloride, sodium citrate (SSC), 50% formamide, 0.1M Tris buffer at pH 7.5, 5X Denhardt's solution; 2) pre-hybridization at 37°C for 4 hours; 3) hybridization at 37°C with amount of labeled probe equal to 3,000,000 cpm total for 16 hours; 4) wash in 2X SSC and 0.1% SDS solution; 5) wash 4X for 1 minute each at room temperature and 4X at 60°C for 30 minutes each; and 6) dry and expose to film.

The phrase "selectively hybridizing to" refers to a nucleic acid molecule that hybridizes, duplexes or binds only to a particular target DNA or RNA sequence when the target sequences are present in a preparation of total cellular DNA or RNA. By selectively hybridizing it is meant that a nucleic acid molecule binds to a given target in a manner that is detectable in a different manner from non-target sequence under moderate, or more preferably under high, stringency conditions of hybridization. "Complementary" or "target" nucleic acid sequences refer to those nucleic acid sequences which selectively hybridize to a nucleic acid molecule. Proper annealing conditions depend, for example, upon a nucleic acid molecule's length, base composition, and the number of mismatches and their position on the molecule, and must often be determined empirically. For discussions of

nucleic acid molecule (probe) design and annealing conditions, see, for example, Sambrook et al. 1989.

It will be readily understood by those skilled in the art and it is intended here, that when reference is made to particular sequence listings, such reference includes sequences which substantially correspond to its complementary sequence and those described including allowances for minor sequencing errors, single base changes, deletions, substitutions and the like, such that any such sequence variation corresponds to the nucleic acid sequence of the signal peptide or other peptide/protein to which the relevant sequence listing relates.

The DNA molecules of the subject invention also include DNA molecules coding for protein analogs, fragments or derivatives of the protein which differ from naturally-occurring forms (the naturally-occurring protein) in terms of the identity or location of one or more amino acid residues (deletion analogs containing less than all of the residues specified for the protein, substitution analogs wherein one or more residues specified are replaced by other residues, and addition analogs wherein one or more amino acid residues is added to a terminal or medial portion of the protein) and which share the signal property of the naturally-occurring form. These molecules include: the incorporation of codons "preferred" for expression by selected non-mammalian hosts; the provision of sites for cleavage by restriction endonuclease enzymes; and the provision of additional initial, terminal or intermediate DNA sequences that facilitate construction of readily expressed vectors.

As used herein, a "peptide" refers to an amino acid sequence of three to one hundred amino acids, and

therefore an isolated peptide that comprises an amino acid sequence is not intended to cover amino acid sequences of greater than 100 amino acids. Preferably, the peptides that can be identified and used in accordance with the subject invention (whether they be mimotope or anti-mimotope peptides) are less than 50 amino acids in length, and more preferably the peptides are five to 20 amino acids in length or 20-40 amino acids in length.

The peptides can contain any naturally-occurring or non-naturally-occurring amino acids, including the D-form of the amino acids, amino acid derivatives and amino acid mimics, so long as the desired function and activity of the peptide is maintained. The choice of including an (L)- or a (D)-amino acid in the peptides depends, in part, on the desired characteristics of the peptide. For example, the incorporation of one or more (D)-amino acids can confer increased stability on the peptide and can allow a peptide to remain active in the body for an extended period of time. The incorporation of one or more (D)-amino acids can also increase or decrease the pharmacological activity of the peptide.

The peptides may also be cyclized, since cyclization may provide the peptides with superior properties over their linear counterparts.

As used herein, the terms "amino acid mimic" and "mimetic" mean an amino acid analog or non-amino acid moiety that has the same or similar functional characteristic of a given amino acid. For instance, an amino acid mimic of a hydrophobic amino acid is one which is non-polar and retains hydrophobicity, generally by way of containing an aliphatic chemical group. By way of further example, an arginine mimic can be an analog of arginine which contains a side chain having a positive

charge at physiological pH, as is characteristic of the guanidinium side chain reactive group of arginine.

In addition, modifications to the peptide backbone and peptide bonds thereof are also encompassed within the scope of amino acid mimic or mimetic. Such modifications can be made to the amino acid, derivative thereof, non-amino acid moiety or the peptide either before or after the amino acid, derivative thereof or non-amino acid moiety is incorporated into the peptide. What is critical is that such modifications mimic the peptide backbone and bonds which make up the same and have substantially the same spacial arrangement and distance as is typical for traditional peptide bonds and backbones. An example of one such modification is the reduction of the carbonyl(s) of the amide peptide backbone to an amine. A number of reagents are available and well known for the reduction of amides to amines such as those disclosed in Wann et al., JOC, 46:257 (1981) and Raucher et al., Tetrahedron. Lett., 21:14061 (1980). An amino acid mimic is, therefor, an organic molecule that retains the similar amino acid pharmacophore groups as is present in the corresponding amino acid and which exhibits substantially the same spatial arrangement between functional groups.

The substitution of amino acids by non-naturally occurring amino acids and amino acid mimics as described above can enhance the overall activity or properties of an individual peptide based on the modifications to the backbone or side chain functionalities. For example, these types of alterations to the specifically described amino acid substituents and exemplified peptides can enhance the peptide's stability to enzymatic breakdown and increase biological activity. Modifications to the

peptide backbone similarly can add stability and enhance activity.

One skilled in the art, using the above sequences or formulae, can easily synthesize the peptides. Standard procedures for preparing synthetic peptides are well known in the art. The novel peptides can be synthesized using: the solid phase peptide synthesis (SPPS) method of Merrifield (J. Am. Chem. Soc., 85:2149 (1964)) or modifications of SPPS; or, the peptides can be synthesized using standard solution methods well known in the art (see, for example, Bodanzsky, M., Principles of Peptide Synthesis, 2nd revised ed., Springer-Verlag (1988 and 1993)). Alternatively, simultaneous multiple peptide synthesis (SMPS) techniques well known in the art can be used. Peptides prepared by the method of Merrifield can be synthesized using an automated peptide synthesizer such as the Applied Biosystems 431A-01 Peptide Synthesizer (Mountain View, Calif.) or using the manual peptide synthesis technique described by Houghten, Proc. Natl. Acad. Sci., USA 82:5131 (1985).

With these definitions in mind, the subject invention provides an isolated nucleic acid molecule encoding a pancreatic T-type calcium channel. The nucleic acid molecule can be deoxyribonucleic acid (DNA) or ribonucleic acid (RNA, including messenger RNA or mRNA), genomic or recombinant, biologically isolated or synthetic.

The DNA molecule can be a cDNA molecule, which is a DNA copy of a messenger RNA (mRNA) encoding the channel.

An example of such a pancreatic T-type calcium channel is the rat pancreatic T-type calcium channel encoded by the nucleotide sequence as shown in SEQ ID NO:1. The amino acid sequence encoded by this nucleotide sequence is shown in SEQ ID NO:2.

The invention also provides an antisense nucleic acid molecule that is complementary to at least a portion of the mRNA encoding the pancreatic T-type calcium channel. Antisense nucleic acid molecules can be RNA or single-stranded DNA, and can be complementary to the entire mRNA molecule encoding the channel (i.e. of the same nucleotide length as the entire molecule). It may be desirable, however, to work with a shorter molecule. In this instance, the antisense molecule can be complementary to a portion of the entire mRNA molecule encoding the channel. These shorter antisense molecules are capable of hybridizing to the mRNA encoding the entire molecule, and preferably consist of about twenty to about one hundred nucleotides. These antisense molecules can be used to reduce levels of pancreatic T-type calcium channel, by introducing into cells an RNA or single-stranded DNA molecule that is complementary to at least a portion of the mRNA of the channel (i.e. by introducing an antisense molecule). The antisense molecule can base-pair with the mRNA of the channel, preventing translation of the mRNA into protein. Thus, an antisense molecule to the channel can prevent translation of mRNA encoding the channel into a functional channel protein. It may be desirable to place the antisense molecule downstream and under the control or the insulin promoter, so that the antisense will prevent translation of mRNA encoding the T type calcium channel only in islet cells of the pancreas (not affecting brain or heart T type calcium channels). It should also be apparent that 100% prevention of T type calcium channel is not desirable, since a minimal basal Ca^{2+} level is required to be maintained by the T type calcium channel.

More particularly, an antisense molecule complementary to at least a portion of mRNA encoding a pancreatic T-type calcium channel can be used to decrease expression of a functional channel. A cell with a first level of expression of a functional pancreatic T-type calcium channel is selected, and then the antisense molecule is introduced into the cell. The antisense molecule blocks expression of functional pancreatic T-type calcium channel, resulting in a second level of expression of a functional pancreatic T-type calcium channel in the cell. The second level is less than the initial first level.

Antisense molecules can be introduced into cells by any suitable means. In one embodiment, the antisense RNA molecule is injected directly into the cellular cytoplasm, where the RNA interferes with translation. A vector may also be used for introduction of the antisense molecule into a cell. Such vectors include various plasmid and viral vectors. For a general discussion of antisense molecules and their use, see Han et al. 1991 and Rossi 1995.

The invention further provides a special category of antisense RNA molecules, known as ribozymes, having recognition sequences complementary to specific regions of the mRNA encoding the pancreatic T-type calcium channel. Ribozymes not only complex with target sequences via complementary antisense sequences but also catalyze the hydrolysis, or cleavage, of the template mRNA molecule. Examples, which are not intended to be limiting, of suitable regions of the mRNA template to be targeted by ribozymes are any of the homologous regions identified by comparing the various T-type calcium channels, and particularly pancreatic β-cell T-type channels.

Expression of a ribozyme in a cell can inhibit gene expression (such as the expression of a pancreatic T-type calcium channel). More particularly, a ribozyme having a recognition sequence complementary to a region of a mRNA encoding a pancreatic T-type calcium channel can be used to decrease expression of pancreatic T-type calcium channel. A cell with a first level of expression of pancreatic T-type calcium channel is selected, and then the ribozyme is introduced into the cell. The ribozyme in the cell decreases expression of pancreatic T-type calcium channel in the cell, because mRNA encoding the pancreatic T-type calcium channel is cleaved and cannot be translated.

Ribozymes can be introduced into cells by any suitable means. In one embodiment, the ribozyme is injected directly into the cellular cytoplasm, where the ribozyme cleaves the mRNA and thereby interferes with translation. A vector may be used for introduction of the ribozyme into a cell. Such vectors include various plasmid and viral vectors (note that the DNA encoding the ribozyme does not need to be "incorporated" into the genome of the host cell; it could be expressed in a host cell infected by a viral vector, with the vector expressing the ribozyme, for instance). For a general discussion of ribozymes and their use, see Sarver et al. 1990, Chrisey et al. 1991, Rossi et al. 1992, and Christoffersen et al. 1995.

The nucleic acid molecules of the subject invention can be expressed in suitable host cells using conventional techniques. Any suitable host and/or vector system can be used to express the pancreatic T-type calcium channel. For in vitro expression, Xenopus oocytes are preferred. For in vivo expression, the most suitable host cell is a pancreatic β-cell.

Techniques for introducing the nucleic acid molecules into the host cells may involve the use of expression vectors which comprise the nucleic acid molecules. These expression vectors (such as plasmids and viruses; viruses including bacteriophage) can then be used to introduce the nucleic acid molecules into suitable host cells. For example, DNA encoding the pancreatic T-type calcium channel can be injected into the nucleus of a host cell or transformed into the host cell using a suitable vector, or mRNA encoding the pancreatic T-type calcium channel can be injected directly into the host cell, in order to obtain expression of pancreatic T-type calcium channel in the host cell.

Various methods are known in the art for introducing nucleic acid molecules into host cells. One method is microinjection, in which DNA is injected directly into the nucleus of cells through fine glass needles (or RNA is injected directly into the cytoplasm of cells). Alternatively, DNA can be incubated with an inert carbohydrate polymer (dextran) to which a positively charged chemical group (DEAE, for diethylaminoethyl) has been coupled. The DNA sticks to the DEAE-dextran via its negatively charged phosphate groups. These large DNA-containing particles stick in turn to the surfaces of cells, which are thought to take them in by a process known as endocytosis. Some of the DNA evades destruction in the cytoplasm of the cell and escapes to the nucleus, where it can be transcribed into RNA like any other gene in the cell. In another method, cells efficiently take in DNA in the form of a precipitate with calcium phosphate. In electroporation, cells are placed in a solution containing DNA and subjected to a brief electrical pulse that causes holes to open transiently in

their membranes. DNA enters through the holes directly into the cytoplasm, bypassing the endocytotic vesicles through which they pass in the DEAE-dextran and calcium phosphate procedures. DNA can also be incorporated into artificial lipid vesicles, liposomes, which fuse with the cell membrane, delivering their contents directly into the cytoplasm. In an even more direct approach, DNA is absorbed to the surface of tungsten microprojectiles and fired into cells with a device resembling a shotgun.

Several of these methods, microinjection, electroporation, and liposome fusion, have been adapted to introduce proteins into cells. For review, see Mannino and Gould-Fogerite 1988, Shigekawa and Dower 1988, Capecchi 1980, and Klein et al. 1987.

Further methods for introducing nucleic acid molecules into cells involve the use of viral vectors. One such virus widely used for protein production is an insect virus, baculovirus. For a review of baculovirus vectors, see Miller (1989). Various viral vectors have also been used to transform mammalian cells, such as bacteriophage, vaccinia virus, adenovirus, and retrovirus.

As indicated, some of these methods of transforming a cell require the use of an intermediate plasmid vector. U.S. Patent No. 4,237,224 to Cohen and Boyer describes the production of expression systems in the form of recombinant plasmids using restriction enzyme cleavage and ligation with DNA ligase. These recombinant plasmids are then introduced by means of transformation and replicated in unicellular cultures including procaryotic organisms and eucaryotic cells grown in tissue culture. The DNA sequences are cloned into the plasmid vector using standard cloning procedures known in the art, as described by Sambrook et al. (1989).

Host cells into which the nucleic acid encoding the pancreatic T-type calcium channel has been introduced can be used to produce (i.e. to functionally express) the pancreatic T-type calcium channel. The function of the encoded pancreatic T-type calcium channel can be assayed according to methods known in the art (Wang et al. 1996).

Having identified the nucleic acid molecules encoding pancreatic T-type calcium channels and methods for expressing the pancreatic T-type calcium channels encoded thereby, the invention further provides a method of screening a substance (for example, a compound or inhibitor) for the ability of the substance to modify T-type calcium channel function. The method comprises introducing a nucleic acid molecule encoding the pancreatic T-type calcium channel into a host cell, and expressing the pancreatic T-type calcium channel encoded by the molecule in the host cell. The cell is then exposed to a substance and evaluated to determine if the substance modifies the function of the T-type calcium channel. From this evaluation, substances effective in altering the function of the T-type calcium channel can be found. Such agents may be, for example, calcium channel inhibitors, agonists, or antagonists (for example, mibefradil and mibefradil analogues, amiloride, $NiCl_2$, antisense molecules, and second messengers).

The evaluation of the cell to determine if the substance modifies the function of the T-type calcium channel can be by any means known in the art. The evaluation can comprise the direct monitoring of expression of T-type calcium channel in the host cell, or the evaluation can be indirect and comprise the monitoring of calcium transport by the channel (such as by the methods disclosed by Wang et al. 1996).

The nucleic acid molecules of the subject invention can be used either as probes or for the design of primers to obtain DNA encoding other pancreatic T-type calcium channels by either cloning and colony/plaque hybridization or amplification using the polymerase chain reaction (PCR).

Specific probes derived from SEQ ID NO:1 can be employed to identify colonies or plaques containing cloned DNA encoding a member of the pancreatic T-type calcium channel family using known methods (see Sambrook et al. 1989). One skilled in the art will recognize that by employing such probes under high stringency conditions (for example, hybridization at 42°C with 5X SSPC and 50% formamide, washing at 50-65°C with 0.5X SSPC), sequences having regions which are greater than 90% homologous or identical to the probe can be obtained. Sequences with lower percent homology or identity to the probe, which also encode pancreatic T-type calcium channels, can be obtained by lowering the stringency of hybridization and washing (e.g., by reducing the hybridization and wash temperatures or reducing the amount of formamide employed).

More particularly, in one embodiment, the method comprises selection of a DNA molecule encoding a pancreatic T-type calcium channel, or a fragment thereof, the DNA molecule having a nucleotide sequence as shown in SEQ ID NO:1, and designing an oligonucleotide probe for pancreatic T-type calcium channel based on SEQ ID NO:1. A genomic or cDNA library of an organism is then probed with the oligonucleotide probe, and clones are obtained from the library that are recognized by the oligonucleotide probe so as to obtain DNA encoding another pancreatic T-type calcium channel.

Specific primers derived from SEQ ID NO:1 can be used in PCR to amplify a DNA sequence encoding a member of the pancreatic T-type calcium channel family using known methods (see Innis et al. 1990). One skilled in the art will recognize that by employing such primers under high stringency conditions (for example, annealing at 50-60°C, depending on the length and specific nucleotide content of the primers employed), sequences having regions greater than 75% homologous or identical to the primers will be amplified.

More particularly, in a further embodiment the method comprises selection of a DNA molecule encoding pancreatic T-type calcium channel, or a fragment thereof, the DNA molecule having a nucleotide sequence as shown in SEQ ID NO:1, designing degenerate oligonucleotide primers based on regions of SEQ ID NO:1, and employing such primers in the polymerase chain reaction using as a template a DNA sample to be screened for the presence of pancreatic T-type calcium channel-encoding sequences. The resulting PCR products can be isolated and sequenced to identify DNA fragments that encode polypeptide sequences corresponding to the targeted region of pancreatic T-type calcium channel.

Various modifications of the nucleic acid and amino acid sequences disclosed herein are covered by the subject invention. These varied sequences still encode a functional pancreatic T-type calcium channel. The invention thus further provides an isolated nucleic acid molecule encoding a pancreatic T-type calcium channel, the nucleic acid molecule encoding a first amino acid sequence having at least 90% amino acid identity to a second amino acid sequence, the second amino acid sequence as shown in SEQ ID NO:2. In further embodiments, the first amino acid sequence has at least

95%, 96%, 97%, 98%, or 99% amino acid identity to SEQ ID NO:2.

The invention further provides an isolated DNA oligomer capable of hybridizing to the nucleic acid molecule encoding pancreatic T-type calcium channel according to the subject invention. Such oligomers can be used as probes in a method of detecting the presence of pancreatic T-type calcium channel in a sample. More particularly, a sample can be contacted with the DNA oligomer and the DNA oligomer will hybridize to any pancreatic T-type calcium channel present in the sample, forming a complex therewith. The complex can then be detected, thereby detecting presence of pancreatic T-type calcium channel in the sample.

The complex can be detected using methods known in the art. Preferably, the DNA oligomer is labeled with a detectable marker so that detection of the marker after the DNA oligomer hybridizes to any pancreatic T-type calcium channel in the sample (wherein non-hybridized DNA oligomer has been washed away) is detection of the complex. Detection of the complex indicates the presence of pancreatic T-type calcium channel in the sample. As will be readily apparent to those skilled in the art, such a method could also be used quantitatively to assess the amount of pancreatic T-type calcium channel in a sample.

For detection, the oligomers can be labeled with, for example, a radioactive isotope, biotin, an element opaque to X-rays, or a paramagnetic ion. Radioactive isotopes are commonly used and are well known to those skilled in the art. Representative examples include indium-111, technetium-99m, and iodine-123. Biotin is a standard label which would allow detection of the biotin labeled oligomer with avidin. Paramagnetic ions are also

commonly used and include, for example, chelated metal ions of chromium (III), manganese (II), and iron (III). When using such labels, the labeled DNA oligomer can be imaged using methods known to those skilled in the art. Such imaging methods include, but are not limited to, X-ray, CAT scan, PET scan, NMRI, and fluoroscopy. Other suitable labels include enzymatic labels (horseradish peroxidase, alkaline phosphatase, etc.) and fluorescent labels (such as FITC or rhodamine, etc.).

The invention further provides an isolated pancreatic T-type calcium channel protein. The protein is preferably encoded by a nucleotide sequence as shown in SEQ ID NO:1. The protein preferably has an amino acid sequence as shown in SEQ ID NO:2. Further provided is an isolated pancreatic T-type calcium channel protein encoded by a first amino acid sequence having at least 90% amino acid identity to a second amino acid sequence, the second amino acid sequence as shown in SEQ ID NO:2. In further embodiments, the first amino acid sequence has at least 95%, 96%, 97%, 98%, or 99% amino acid identity to SEQ ID NO:2.

The pancreatic T-type calcium channel molecule of the subject invention can include a leader sequence for targeting of the pancreatic T-type calcium channel protein to the desired part of a cell.

It should be readily apparent to those skilled in the art that a met residue may need to be added to the amino terminal of the amino acid sequence of the mature pancreatic T-type calcium channel protein (i.e., added to SEQ ID NO:2) or an ATG added to the 5' end of the nucleotide sequence (i.e., added to SEQ ID NO:1), in order to express the channel in a host cell. The met version of the mature channel is thus specifically

intended to be covered by reference to SEQ ID NO:1 or SEQ ID NO:2.

The invention further provides an antibody or fragment thereof specific for the pancreatic T-type calcium channel of the subject invention. Antibodies of the subject invention include polyclonal antibodies and monoclonal antibodies capable of binding to the pancreatic T-type calcium channel, as well as fragments of these antibodies, and humanized forms. Humanized forms of the antibodies of the subject invention may be generated using one of the procedures known in the art such as chimerization. Fragments of the antibodies of the present invention include, but are not limited to, the Fab, the $F(ab')_2$, and the Fc fragments.

The invention also provides hybridomas which are capable of producing the above-described antibodies. A hybridoma is an immortalized cell line which is capable of secreting a specific monoclonal antibody.

In general, techniques for preparing polyclonal and monoclonal antibodies as well as hybridomas capable of producing the desired antibody are well known in the art (see Campbell 1984 and St. Groth et al. 1980). Any animal (mouse, rabbit, etc.) which is known to produce antibodies can be immunized with the antigenic pancreatic T-type calcium channel (or an antigenic fragment thereof). Methods for immunization are well known in the art. Such methods include subcutaneous or intraperitoneal injection of the protein. One skilled in the art will recognize that the amount of the protein used for immunization will vary based on the animal which is immunized, the antigenicity of the protein, and the site of injection.

The protein which is used as an immunogen may be modified or administered in an adjuvant in order to

increase the protein's antigenicity. Methods of increasing the antigenicity of a protein are well known in the art and include, but are not limited to, coupling the antigen with a heterologous protein (such as a globulin or beta-galactosidase) or through the inclusion of an adjuvant during immunization.

For monoclonal antibodies, spleen cells from the immunized animals are removed, fused with myeloma cells, such as SP2/O-Ag 15 myeloma cells, and allowed to become monoclonal antibody producing hybridoma cells.

Any one of a number of methods well known in the art can be used to identify the hybridoma cell which produces an antibody with the desired characteristics. These include screening the hybridomas with an ELISA assay, western blot analysis, or radioimmunoassay (Lutz et al. 1988).

Hybridomas secreting the desired antibodies are cloned and the class and subclass are determined using procedures known in the art (Campbell 1984).

For polyclonal antibodies, antibody containing antisera is isolated from the immunized animal and is screened for the presence of antibodies with the desired specificity using one of the above-described procedures.

The present invention further provides the above-described antibodies in detectably labeled form. Antibodies can be detectably labeled through the use of radioisotopes, affinity labels (such as biotin, avidin, etc.), enzymatic labels (such as horseradish peroxidase, alkaline phosphatase, etc.), fluorescent labels (such as FITC or rhodamine, etc.), paramagnetic atoms, etc. Procedures for accomplishing such labeling are well known in the art, for example see Sternberger et al. 1970, Bayer et al. 1979, Engval et al. 1972, and Goding 1976.

The labeled antibodies or fragments thereof of the present invention can be used for in vitro, in vivo, and in situ assays to identify cells or tissues which express pancreatic T-type calcium channel, to identify samples containing pancreatic T-type calcium channel, or to detect the presence of pancreatic T-type calcium channel in a sample. More particularly, the antibodies or fragments thereof can thus be used to detect the presence of pancreatic T-type calcium channel in a sample, by contacting the sample with the antibody or fragment thereof. The antibody or fragment thereof binds to any pancreatic T-type calcium channel present in the sample, forming a complex therewith. The complex can then be detected, thereby detecting the presence of pancreatic T-type calcium channel in the sample. As will be readily apparent to those skilled in the art, such a method could also be used quantitatively to assess the amount of pancreatic T-type calcium channel in a sample. As should also be readily apparent, such an antibody can also be used to decrease levels of functional T type calcium channels, by blocking the channel. Such antibodies can therefore be used in the methods of the subject invention to modify levels of functional T type calcium channels in pancreatic beta cells.

Further provided is a composition comprising the pancreatic T-type calcium channel protein and a compatible carrier.

In the methods of the invention, tissues or cells are contacted with or exposed to the composition of the subject invention or a compound. In the context of this invention, to "contact" tissues or cells with or to "expose" tissues or cells to a composition or compound means to add the composition or compound, usually in a liquid carrier, to a cell suspension or tissue sample,

either in vitro or ex vivo, or to administer the composition or compound to cells or tissues within an animal (including humans).

For therapeutics, methods of modifying insulin secretion by pancreatic beta cells, methods of treating type II diabetes, methods of modifying basal calcium levels in cells, methods of modifying the action potential of L type calcium channels in cells, methods of modifying pancreatic beta cell death, methods of modifying pancreatic beta cell proliferation, and methods of modifying calcium influx through L type calcium channels in cells, each of the methods comprising modifying levels of functional T type calcium channels in the cells, are provided. The formulation of therapeutic compositions and their subsequent administration is believed to be within the skill in the art. In general, for therapeutics, a patient suspected of needing such therapy is given a composition in accordance with the invention, commonly in a pharmaceutically acceptable carrier, in amounts and for periods which will vary depending upon the nature of the particular disease, its severity and the patient's overall condition. The pharmaceutical compositions of the present invention may be administered in a number of ways depending upon whether local or systemic treatment is desired and upon the area to be treated. Administration may be topical (including ophthalmic, vaginal, rectal, intranasal, transdermal), oral or parenteral. Parenteral administration includes intravenous drip or infusion, subcutaneous, intraperitoneal or intramuscular injection, pulmonary administration, e.g., by inhalation or insufflation, or intrathecal or intraventricular administration.

Formulations for topical administration may include transdermal patches, ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders. Conventional pharmaceutical carriers, aqueous, powder or oily bases, thickeners and the like may be necessary or desirable. Coated condoms, gloves and the like may also be useful.

Compositions for oral administration include powders or granules, suspensions or solutions in water or non-aqueous media, capsules, sachets or tablets. Thickeners, flavoring agents, diluents, emulsifiers, dispersing aids or binders may be desirable.

Compositions for parenteral, intrathecal or intraventricular administration may include sterile aqueous solutions which may also contain buffers, diluents and other suitable additives.

In addition to such pharmaceutical carriers, cationic lipids may be included in the formulation to facilitate uptake. One such composition shown to facilitate uptake is LIPOFECTIN (BRL, Bethesda MD).

Dosing is dependent on severity and responsiveness of the condition to be treated, with course of treatment lasting from several days to several months or until a cure is effected or a diminution of disease state is achieved. Optimal dosing schedules can be calculated from measurements of drug accumulation in the body. Persons of ordinary skill can easily determine optimum dosages, dosing methodologies and repetition rates. Optimum dosages may vary depending on the relative potency of individual compositions, and can generally be calculated based on IC_{50}'s or EC_{50}'s in in vitro and in vivo animal studies. For example, given the molecular weight of compound (derived from oligonucleotide sequence and/or chemical structure) and an effective dose such as

an IC_{50}, for example (derived experimentally), a dose in mg/kg is routinely calculated.

The methods of the subject invention are based on the discovery that regulation of T type calcium channels directly modifies basal calcium levels in cells, which in turn regulates L type calcium channel activity, which in turn regulates insulin secretion and cell death, which in turn treats type II diabetes. The methods of the subject invention are further based on the discovery that regulation of T type calcium channels directly affects basal and glucose-induced insulin secretion.

T type calcium channels belong to the family of low voltage activated calcium channels. Modifying (increasing or decreasing) "levels" of functional T type calcium channels refers to modifying expression of the T type calcium channel gene, modifying activity of the T type calcium channel such as by inhibiting the function of the channel, and/or modifying the formation of active membrane-spanning T type calcium channels. As used herein, "functional" refers to the synthesis and any necessary post-translational processing of a calcium channel molecule in a cell so that the channel is inserted properly in the cell membrane and is capable of conducting calcium ions in accordance with a low voltage activated channel.

The invention thus provides a method of modifying insulin secretion by pancreatic beta cells, the method comprising modifying levels of T type calcium channels in the pancreatic beta cells.

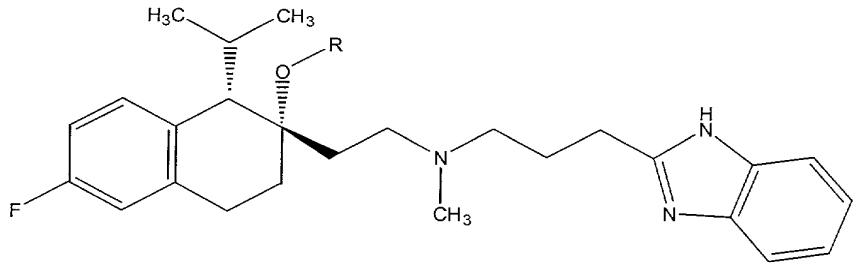
Levels of T type calcium channels in the pancreatic beta cells can be modified by various methods, at the gene and protein and "functional calcium channel" levels. In one embodiment, the levels are modified by modifying T type calcium channel gene expression of the T type

calcium channel in the cells. This can be accomplished by exposing the cells to a compound which modifies T type calcium channel gene expression of the calcium channel. The compound could be, for example, an antisense oligonucleotide targeted to the T type calcium channel gene. In a similar embodiment, the compound which modifies T type calcium channel gene expression of the T type calcium channel could be a ribozyme.

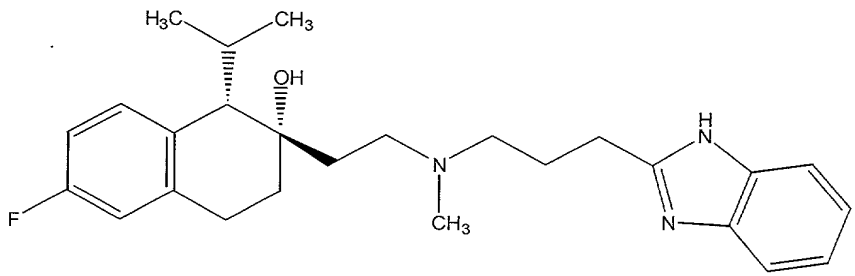
Other methods for modifying T type calcium channel gene expression could also involve site-directed mutagenesis of the T type calcium channel gene to prevent expression of the T type calcium channel, or various gene therapy techniques.

Levels, in particular activity, of T type calcium channels in the cell can also be modified by exposing the cells to an inhibitor of the T type calcium channel. Such inhibitors include, for example, mibefradil, mibefradil analogs, amiloride, $NiCl_2$, and second messengers which regulate activity of the T type calcium channels. Other inhibitors of the T type calcium channel could also readily be identified by screening methods (including the method described above). In addition to chemical inhibitors, peptide inhibitors could also be identified with screening methods (for example, using phage display libraries and other peptide screening methods).

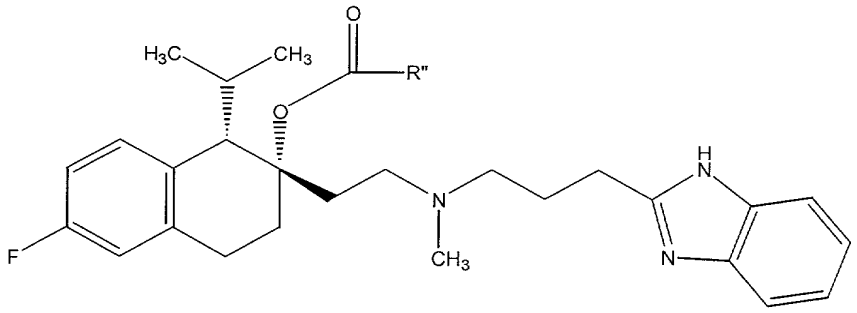
"Mibefradil analogs", as used herein are meant to include compounds having the formula:



wherein R is hydrogen, alkyl, or a moiety having the formula C(O)R', where R' is alkyl or aryl. In the above formulae, alkyl is meant to include linear alkyls, particularly C1-C12 linear alkyls (e.g., methyl, ethyl, n-propyl, n-pentyl, n-hexyl, n-heptyl, n-octyl, n-nonyl, and the like), branched alkyls, particularly C1-C12 branched alkyls (e.g., isobutyl, isopentyl, neopentyl, hex-2-yl, hex-3-yl, hept-2-yl, hept-3-yl, and the like), and cycloalkyls, particularly C1-C8 cycloalkyls (e.g., cyclopentyl, cyclohexyl, cycloheptyl, 4-methylcyclohexyl, and the like). These alkyl groups can be substituted or unsubstituted. When substituted, suitable substituents include, for example, aryl groups, halogen atoms, hydroxy groups, alkoxy groups, carboxylic acid groups, amine groups, and the like, as well as combinations of these substituents. Mibefradil analogs which are particularly well suited to blocking (inhibiting) the activity of T-type calcium channels but not blocking the activity of L-type calcium channels are those having the formula:



and those having the formula:



in which R" is an unsubstituted alkyl group or a substituted alkyl group which does not contain an alkoxy substituent. "Mibefradil analogs" are also meant to include compounds having the above formulae which are substituted at other positions in the structure, for example, on the benzimidazole phenyl moiety, at a benzimidazole nitrogen, at other positions of the tetrahydronaphthyl ring, etc. Also included within the meaning of "mibefradil analogs" are compounds having the above formulae in which the F is replaced with another substituent, such as another halogen. Also included within the meaning of "mibefradil analogs" are compounds having the above formulae in which the amine methyl group or the isopropyl group or both are replaced with other substituents, such as other alkyl moieties. Additionally, "mibefradil analogs" are meant to include those compounds which are generically described and/or specifically disclosed in U.S. Patent No. 4,808,605, which is hereby incorporated by reference. Further, "mibefradil analogs" are meant to include pharmaceutically acceptable salts of the derivatives described above. Illustrative pharmaceutically acceptable salts are salts formed with hydrochloric acid, hydrobromic acid, nitric acid, sulphuric acid, phosphoric acid, citric acid, formic acid, maleic acid, acetic acid,

succinic acid, tartaric acid, methanesulphonic acid, p-toluenesulphonic, and the like.

Mibefradil analogs can be made by following the general procedures described in, for example, U.S. Patent Nos. 4,808,605, 5,910,606, 5,892,055, 5,811,557, 5,811,556, and 5,808,088, each of which is hereby incorporated by reference.

Levels of T type calcium channels in the cell can also be modified by exposing the cells to a compound which interferes with membrane T type calcium channel formation.

Levels of functional T type calcium channel could also be modified by use of molecules which bind to transcription regulators of the T type calcium channel gene (such as the promoter region of the gene).

The invention further provides a method of treating type II diabetes in a subject (human or animal), the method comprising administering to the subject an amount of a compound effective to modify levels of T type calcium channels in the pancreatic beta cells of the subject. As above, the compound may modify levels of T type calcium channels by modifying T type calcium channel gene expression of the calcium channel, or by inhibiting the T type calcium channel, or by interfering with membrane T type calcium channel formation.

In the context of this invention "modulation" or "modifying" means either inhibition or stimulation. This modulation can be measured in ways which are routine in the art, for example by Northern blot assay of mRNA expression, Western blot assay of protein expression, or calcium channel activity assay.

The compounds and/or inhibitors used in the methods of the subject invention encompass any pharmaceutically acceptable salts, esters, or salts of such esters, or any

other compound/inhibitor which, upon administration to an animal including a human, is capable of providing (directly or indirectly) the biologically active metabolite or residue thereof. Accordingly, for example, the disclosure is also drawn to prodrugs and pharmaceutically acceptable salts of the compounds and/or inhibitors used in the subject invention, pharmaceutically acceptable salts of such prodrugs, and other bioequivalents.

In regard to prodrugs, the compounds and/or inhibitors for use in the invention may additionally or alternatively be prepared to be delivered in a prodrug form. The term prodrug indicates a therapeutic agent that is prepared in an inactive form that is converted to an active form (i.e., drug) within the body or cells thereof by the action of endogenous enzymes or other chemicals and/or conditions.

In regard to pharmaceutically acceptable salts, the term pharmaceutically acceptable salts refers to physiologically and pharmaceutically acceptable salts of the compounds and/or inhibitors used in the subject invention: i.e., salts that retain the desired biological activity of the parent compound and do not impart undesired toxicological effects thereto.

Drugs, such as peptide drugs, which inhibit the T type calcium channel or which interfere with functional T type calcium channel formation can be identified by other methods also. For example, a monoclonal antibody can be prepared which specifically hybridizes to the T type calcium channel, thereby interfering with activity and/or channel formation. Once a monoclonal antibody which specifically hydridizes to the T type calcium channel is identified, the monoclonal (which is itself a compound or inhibitor which can be used in the subject invention) can

be used to identify peptides capable of mimicking the inhibitory activity of the monoclonal antibody. One such method utilizes the development of epitope libraries and biopanning of bacteriophage libraries. Briefly, attempts to define the binding sites for various monoclonal antibodies have led to the development of epitope libraries. Parmley and Smith developed a bacteriophage expression vector that could display foreign epitopes on its surface (Parmley, S.F. & Smith, G.P., Gene 73:305-318 (1988)). This vector could be used to construct large collections of bacteriophage which could include virtually all possible sequences of a short (e.g. six-amino-acid) peptide. They also developed biopanning, which is a method for affinity-purifying phage displaying foreign epitopes using a specific antibody (see Parmley, S.F. & Smith, G.P., Gene 73:305-318 (1988); Cwirla, S.E., et al., Proc Natl Acad Sci USA 87:6378-6382 (1990); Scott, J.K. & Smith, G.P., Science 249:386-390 (1990); Christian, R.B., et al., J Mol Biol 227:711-718 (1992); Smith, G.P. & Scott, J.K., Methods in Enzymology 217:228-257 (1993)).

After the development of epitope libraries, Smith et al. then suggested that it should be possible to use the bacteriophage expression vector and biopanning technique of Parmley and Smith to identify epitopes from all possible sequences of a given length. This led to the idea of identifying peptide ligands for antibodies by biopanning epitope libraries, which could then be used in vaccine design, epitope mapping, the identification of genes, and many other applications (Parmley, S.F. & Smith, G.P., Gene 73:305-318 (1988); Scott, J.K., Trends in Biochem Sci 17:241-245 (1992)).

Using epitope libraries and biopanning, researchers searching for epitope sequences found instead peptide

sequences which mimicked the epitope, i.e., sequences which did not identify a continuous linear native sequence or necessarily occur at all within a natural protein sequence. These mimicking peptides are called mimotopes. In this manner, mimotopes of various binding sites/proteins have been found.

The sequences of these mimotopes, by definition, do not identify a continuous linear native sequence or necessarily occur in any way in a naturally-occurring molecule, i.e. a naturally occurring protein. The sequences of the mimotopes merely form a peptide which functionally mimics a binding site on a naturally-occurring protein.

Many of these mimotopes are short peptides. The availability of short peptides which can be readily synthesized in large amounts and which can mimic naturally-occurring sequences (i.e. binding sites) offers great potential application.

Using this technique, mimotopes to a monoclonal antibody that recognizes T type calcium channels can be identified. The sequences of these mimotopes represent short peptides which can then be used in various ways, for example as peptide drugs that bind to T type calcium channels and decrease the activity of T type calcium channels. Once the sequence of the mimotope is determined, the peptide drugs can be chemically synthesized.

MATERIALS AND METHODS

Cell Culture - INS-1 cells were cultured in RPMI 1640 medium containing 10% FBS, 25 U/ml penicillin, 25 mg/ml streptomycin and 50 μM mercaptoethanol in an atmosphere of 5% CO_2 in air, at 37°C for 2-5 days before recording.

Islet cell preparation - Pancreases of Sprague-Dawley rats (Charles River Laboratory, Wilmington, MA) were removed after intrapancreatic perfusion with 2 ml of Hanks' solution (Gibco BRL, Grand Island, NY) containing collagenase (4 mg/ml, Boehringer Mannheim, Indianapolis, IN), DNase I (10 μg/ml, Sigma, St. Louis, MO), $CaCl_2$ (1.28 mM) and bovine serum albumin (1 mg/ml, Gibco BRL). The pancreatic tissue was incubated at 37°C for 20 min and then washed five times with enzyme-free Hanks' solution. Islets were picked up and treated with 0.1% pancreatin (Sigma) for five minutes at 37°C. Single cells were obtained by triturating the islets with plastic pipette tips and then they were transferred into 35 mm culture dishes. Cells were cultured in RPMI 1640 medium (Gibco BRL) containing 5 mM glucose, 10% FBS and P/S at 37°C, 5% CO_2 for 2-5 days before experiments.

Isolation of RNA - Total RNA was isolated from cultured INS-1 cells and from various freshly excised rat tissues by the guanidinium isothiocyanate/phenol procedure (Chomczynsk and Sacchi 1987). Poly-A RNA was isolated from total RNA by two successive passes over an oligo (dT)-cellulose spin column (Ambion, Austin, TX).

Cloning of cDNA Encoding α1 Subunit of T-type Ca^{2+} channel in INS-1 - First strand cDNA was prepared using 2 μg of INS-1 cell mRNA and M-MLV reverse transcriptase (Gibco BRL) with the poly-dT primers. The first 433 bp DNA fragment of the channel was deduced with PCR using the degenerate primers (forward)(SEQ ID NO:6) 5'-TNGC(A/C/T)ATGGAG(C/A)GNCC(C/T)-3' and (backward)(SEQ ID NO:7) 5'-CTT(C/G/T)CCCTTGAA(G/C)A(G/A)CTG)-3' based on conserved voltage-dependent Ca^{2+} channel α_1 subunit sequences in domain III. Using the Marathon™ cDNA Amplification Kit (Clontech, Palo Alto, CA), the 3'- and

5'- rapid amplifications of cDNA end-PCR (RACE-PCR) were performed to obtain the entire gene of the α_1 subunit of the channel. For the 5'-RACE-PCR, the forward primer was an adapter primer, the backward primer was (SEQ ID NO:8) 5'-CCGCTGTCGGAGACCATGGAGACC-3'; for the 3'-RACE, the forward primer was (SEQ ID NO:9) 5'-AGCGGCCCAAAATTGACCCCCACAG-3' and the backward primer was poly-dT. The RT-PCR products were subcloned into pT-Adv Vector (Clontech) and dideoxynucleotide sequencing assay was performed with a dsDNA Cycle Sequencing System (Gibco BRL).

Tissue distribution - The gene expression of T-type Ca^{2+} channels deduced from β-cells was examined in rat brain, heart, kidney, and liver using an RT-PCR assay. The primers used for the RT-PCR were (SEQ ID NO:10) 5'-GAAGATGCGAGTGGACAG-3' (forward) and (SEQ ID NO:11) 5'-CTGTGGCGATGGTCACTG-3' (backward). The PCR products were detected by agarose gel electrophoresis on a 1% gel.

Genome walking - The genome walker library (Clontech) was used as a template in nested PCR reactions with gene-specific primers (GSP) and the adapter primers (AP) provided with the kit. The first PCR reaction was carried out in 5 tubes, each having a total volume of 50μl: 5μl 10X PCR reaction buffer, 1 μl dNTP (10 mM each), 2.2μl $Mg(OAc)_2$ (25 mM), 1 μl AP1 (10 μM), 1 μl GSP1, 1 μl Advantage Genomic Polymerase Mix (50X), and 37.8 μl water. The following two-step cycle parameters were used: (Step 1) 7 cycles of denaturing at 94°C for 25 sec., annealing and extension at 72°C for 4 min. (Step 2) 32 cycles of denaturing at 94°C for 25 sec., annealing and extension at 67°C for 4 min. After the second step cycle, the samples were held at 67°C for 4 min. The second PCR reaction was carried out under the reaction condition similar to the first PCR reaction except using

AP2, GSP2. In addition, the templates used were 1 μl of 1:50 dilution of each primary PCR reaction. The two step cycles were similar to the first PCR reaction except 5 cycles at the first step and 22 cycles at the second step.

Oocyte electrophysiology - cRNA transcripts were synthesized from BssH II linearized pT-Adv cDNA templates using T7 RNA polymerase (Ambion). Defolliculated Xenopus laevis were injected with 25 ng pT-Adv cRNA. Three to five days after injection, two-electrode voltage-clamp recording was performed using a Warner OC-725C amplifier (Warner Instrument Corp., Hamden, CT). Data were acquired and analyzed with Pulse/PulseFit software (HEKA, Lambrecht/Pfalz, Germany). The bath solution contained the following: 40 mM $Ca(OH)_2$, 50 mM NaOH, 2 mM TEA-Cl, 1mM KOH, 0.1 mM EDTA and 5 mM HEPES, adjusted to pH 7.4 with methanesulphonate. Boltzmann fits were calculated using Prism (GraphPad). Results are presented as mean ± s.d. unless otherwise stated.

β-cell Electrophysiological recording - The whole-cell recordings were carried out by the standard "giga-seal" patch clamp technique (Hamill et al.). The whole-cell recording pipettes were made of hemocapillaries (Warner), pulled by a two-stage puller (PC-10, Narishige International, New York, NY), and heat polished with a microforge (MF200-1, World Precision Instruments, Sarasota, FL) before use. Pipette resistance was in the range of 2-5 MΩ in the internal solution. The recordings were performed at room temperature (22-25°C). Currents were recorded using an EPC-9 patch-clamp amplifier (HEKA) and filtered at 2.9 kHz. Data were acquired with Pulse/PulseFit software (HEKA). Voltage-dependent currents were corrected for linear leak

and residual capacitance by using an on-line P/n subtraction paradigm.

Drugs - Mibefradil ((1S,2S)-2-[2-[[3-(2-Benzimidazolyl)propyl]methyl-amino]ethyl]-6-fluoro-1,2,3,4-tetrahydro-1-isopropyl-2-napthyl methoxy-acetate dihydrochloride) was kindly provided by Dr. J.-P. Clozel (Hoffmann LaRoche, Basel, Switzerland), and can be synthesized according to the methods disclosed in U.S. Patent Nos. 5,892,055, 5,811,557, 5,811,556, and 5,808,088. U.S. Patent No. 4,808,605 describes mibefradil compounds suitable for use in the subject invention.

The free alcohol Des-methoxyacetyl mibefradil (1S,2S)-2-[2-[[3-(2-Benzimidazolyl)propyl] methylamino]ethyl]-6-fluoro-1,2,3,4-tetrahydro-1-isopropyl-2-napthyl hydroxy hydrochloride) was prepared by alkaline hydrolysis: 14.2 mg mibefradil hydrochloride was dissolved in 4 ml methanol + 1 ml 10 N aqueous sodium hydroxide mixture (5 mM was the final concentration of mibefradil). The solution was warmed in a boiling water bath for 10 min. The reaction was followed by mass spectrometry. Upon completion of the hydrolysis, as determined from the mass spectra, the solution was neutralized with 5 M aqueous hydrochloric acid. The slight loss of methanol that occurred by evaporation during the reaction was corrected by adding water to keep the total volume of 5 ml.

Solutions - The extracellular solution used in whole-cell Ca^{2+} current recording contained (in mM): 10 $CaCl_2$, 110 tetraethylammonium-Cl (TEA-Cl), 10 CsCl, 10 N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES), 40 sucrose, 0.5 3,4-diaminopyridine, pH 7.3. The intracellular solution contained (in mM): 130 N-methyl-D-glucamine, 20 EGTA (free acid), 5 bis (2-aminophenoxy)

ethane-N, N, N', N'-tetraacetate (BAPTA), 10 HEPES, 6 $MgCl_2$, 4 $Ca(OH)_2$, pH was adjusted to 7.4 with methanesulfonate. 2 mM Mg-ATP was included in the pipette solution to minimize rundown of L-type Ca^{2+} currents. For Perforated-patch recording, the extracellular solution contained (in mM): 26 Sucrose, 30 TEA-Cl, 10 HEPES, 5 KCl, 2 $CaCl_2$, $MgCl_2$, pH 7.3. The pipette solution contained (in mM): 65 CsOH, 65 CsMS, 20 sucrose, 10 HEPES, 10 $MgCl_2$, 1 $Ca(OH)_2$, pH 7.4.

Mass Spectrometric Analysis - A VG 70-250 SEQ instrument (VG Analytical, Manchaster, UK) was used with fast atom bombardment (FAB) ionization mode to obtain mass spectra of the mibefradil and dm-mibefradil. Cultured INS-1 cells were treated with 20 μM mibefradil for various lengths of time under each experimental condition. The cell pellets were collected after washing three times with PBS and resuspended in 0.5 ml media for mass spectrometric analysis. For a 50 μl cell sample, 20 μl internal standard solution (40 μM verapamil, MW:454) and 5 μl glycerol was added, and 4 μl of this mixture was used for FAB-MS. Several positive ion spectra were recorded in the mass range m/z 750-100 at a mass resolution of 1000, and a scan speed of 2 second/decade. For mibefradil, m/z 496 was the dominant ion $(M+H)^+$ accompanied with a less intense sodiated molecular ion m/z 518. The concentrations of the mibefradil and hydrolyzed mibefradil were obtained by comparing the intensities of m/z 496 and 424 were to the intensity of m/z 455. For calibration, a standard solution of 50 μM drug was subjected to mass spectrometric analysis.

Separation of cytosolic and membrane components - After washing out mibefradil from the bath solution, the cells were collected and the membranes were broken down by vortexing the cells in a solution containing 5% acetic

acid/CH_3CN. The mixture was then spun and the supernatant collected and defined as non-membrane associated components. Pellets were re-suspended in 5 x volume of NaOH (10 N):methanol (1:7) solution at 37°C for 5 min. The mixture was neutralized with 0.5 M HCl and spun down. The remaining pellet and the supernatant were collected separately.

Statistics - All data is presented as mean ± s.d. and the student's t-test was used to calculate p values where given.

EXAMPLE I

Identification and Cloning of a Pancreatic T-type Calcium Channel

The subject invention provides a cDNA encoding a T-type Ca^{2+} channel α_1 subunit derived from the rat insulin secreting cell line, INS-1, which has been identified and sequenced. The sequence of the cDNA indicates a protein composed of 2288 amino acids (SEQ ID NO:2), sharing 96.3% identity to the neuronal T-type Ca^{2+} channel α_1 subunit (α_1G). The transmembrane domains of the protein are highly conserved but the isoform contains three distinct regions as well as 10 single amino acid substitutions in other regions. Sequencing rat genomic DNA revealed that this is an alternative splice isoform of α_1G. Using specific primers and reverse transcription polymerase chain reaction (RT-PCR) it was demonstrated that both splice variants are expressed in rat islets. The isoform deduced from INS-1 was also expressed in brain, neonatal heart and kidney. Functional expression of this α_1G isoform in Xenopus oocytes generated low-voltage activated Ca^{2+} currents. These results provide the molecular biological basis for studies of function of

T-type Ca^{2+} channels in β-cells where these channels play critical roles in diabetes.

The cloning and tissue distribution of an isoform of the T-type Ca^{2+} channel (α_1G-INS) derived from the rat insulin-secreting cell line, INS-1 (Asfari et al. 1992), is described further below.

Based on the conserved amino acid sequence comprising the six transmembrane segments in repeat III of the previously cloned α_1-subunit (Stea et al. 1995), degenerate primers were designed to deduce the cDNA sequence of voltage-dependent Ca^{2+} channel from INS-1 which expresses a high level of T-type Ca^{2+} current (Bhattacharjee et al. 1997). A 433 base pair (bp) DNA fragment was obtained. The rapid amplification of cDNA ends (RACE) strategy was then used to obtain the entire sequence of the channel. The full length cDNA (SEQ ID NO:1) encodes a protein containing 2288 amino acids (SEQ ID NO:2).

The T-type Ca^{2+} channel gene deduced from β-cells shares 96.3% amino acid identity with α_1G, the neuronal isoform of T-type Ca^{2+} channel (Perez-Reyes et al. 1998). The four intramolecular homologous transmembrane domains of β-cell T-type Ca^{2+} channel α_1 subunit are identical (except glycine 1667) to α_1G, with each repeat containing six putative membrane-spanning regions (S1-S6) and a pore-forming region (P-loop). The other highly conserved region is located at the intracellular loop between repeat I and II, where a section of histidine-rich chain is present in the β-cell derived T-type Ca^{2+} channel gene as well as in neuronal and cardiac T-type Ca^{2+} channel genes. This structure in the $loop_{I-II}$ has not been observed in the protein sequences of known high voltage activated Ca^{2+} channels.

In addition to the single amino acids that differ from α_1G, the T-type Ca^{2+} channel gene derived from β-cells contains three unique regions that differ from the amino acid sequence of α_1G. These regions are located at the N-terminal amino acids (aa1-34 of SEQ ID NO:2), intracellular loop I_{II-III} (aa971-994 of SEQ ID NO:2) and intracellular loop L_{III-IV} (aa1570-1588 of SEQ ID NO:2).

Although the amino acid sequence of the deduced channel is entirely different from the α_1G in the N-terminal region (aa1-34 of SEQ ID NO:2), the nucleotide sequences at this region are almost identical except for 4 single nucleotide insertions which are shown in Fig. 1A. These four single nucleotide insertions determine a different start codon as well as those of the amino acid sequences.

To resolve the relationship between the T-type Ca^{2+} channel isoform deduced from INS-1 and α_1G, a section of Sprague-Dawley rat genomic DNA sequence containing the introns and exons between 4845 and 5256 was identified. As shown in Fig. 1B, an exon was found that encodes the α_1G fragment SKEKQMA (SEQ ID NO:5) as well as an exon that encodes fragment 4869-4922 of the INS-1 variant. This region also contains 8.5 kilobases (kb) of intron sequence. Thus, the T-type Ca^{2+} channel α_1 subunit cloned from INS-1 and α_1G are alternative splice isoforms of the same gene.

The genomic DNA sequence was also used to examine the two nucleotide discrepancy between the α_1G cDNA and the isoform cloned from INS-1. The data show that the genomic nucleotide sequence encoding amino acid 1667 is GGC (glycine), which is the same as the cDNA of α_1 subunit cloned from INS-1 and the corresponding residue in α_1H, but is different from α_1G (GCG, alanine). Also of note, there are nine additional single amino acid substitutions

in the isoform deduced from INS-1 as compared to the α_1G. Six correspond to the amino acids found in the analogous position of α_1H: cysteine 1088, glycine 1667, alanine 1700, aspartic acid 1735, threonine 1812, and leucine 1813.

In regard to tissue distribution of T-type Ca^{2+} channels deduced from β-cells and from neurons, expression of the β-cell T-type Ca^{2+} channel was found in rat brain, heart and kidney, but was absent from liver. Both α_1G and the splice form were detected in rat islets and INS-1 cell preparations using RT-PCR. No α_1H was detected.

Functional expression of the T-type Ca^{2+} channels deduced from β-cells has been conducted in Xenopus oocytes using a double-electrode voltage-clamp method. In a solution containing 40 mM Ca^{2+}, a family of current traces representing T-type Ca^{2+} current characteristics were obtained (Fig. 2A). The current slowly activated at -40 mV and peaked at -10 mV. The analysis of time constants of activation and inactivation are shown in Fig. 2B. The voltage-dependent activation (Fig. 2C) and steady-state inactivation (Fig. 2D) were fitted with Boltzmann equation. The calculated $V_{1/2}$'s were -23.8 mV and -45.6 mV for activation and inactivation, respectively; and k 's were 5.3 and -6.0 for activation and inactivation, respectively.

The nucleotide cDNA (SEQ ID NO:1) and amino acid (SEQ ID NO:2) sequences of rat pancreatic T-type calcium channel were determined. SEQ ID NO:3 is the nucleotide sequence beyond the coding region, while SEQ ID NO:4 includes SEQ ID NO:2.

EXAMPLE II

Characterization of the T type Calcium Channel in Relation to Diabetes

Glucose stimulated insulin release is Ca^{2+} dependent process, involving closure of the ATP-sensitive potassium channels, depolarization and opening of the voltage-dependent Ca^{2+} channels. At glucose concentrations below 3 mM, which do not elicit insulin secretion, β-cells are electrically silent with a resting membrane potential of about -70 mV. Raising external glucose produces a slow depolarization, the extend dependent upon the glucose concentration. At glucose levels which elicit insulin release (>7 mM) depolarization is sufficient to reach the threshold potential (-50 mV) at which electrical activity is initiated.

A simple model for glucose-stimulated insulin secretion is summarized in Fig. 12. The resting membrane potential of β-cells is principally determined by the activity of the K-ATP channel. When plasma glucose rises, its uptake and rate of metabolism by β-cells are stimulated. As a consequence, the intracellular ATP (or ATP:ADP ratio) increases which leads to the closure of K-ATP channels and membrane depolarization. This results in the activation of voltage dependent Ca^{2+} channels (T-type and L-type) and the initiation of electrical activity. The increased calcium influx leads to a rise in $[Ca^{2+}]_i$ and consequently insulin secretion.

Rat and human pancreatic β-cells are equipped with L-type and T-type Ca^{2+} channels. The physiological function of T-type Ca^{2+} channels in β-cells insulin-secretion has been demonstrated. These channels facilitate exocytosis by enhancing electrical activity in these cells. L-type and T-type Ca^{2+} channels, under normal conditions, work in concert promoting the rise in

$[Ca^{2+}]_i$ during glucose-stimulated insulin secretion. In β-cells, over-expressed T-type Ca^{2+} channels are, at least in part, responsible for the hyper-responsiveness of insulin secretion to non-glucose depolarizing stimuli in GK rat, and in rat with NIDDM induced by neonatal injection of streptozotocin. However, over-expressed T-type calcium channels over time will ultimately lead to an elevation of basal Ca^{2+} through its window current properties. Therefore, there is a dual effect of T-type Ca^{2+} channels in β-cells depending upon channel number and membrane potential.

Pharmacologically antagonizing T-type calcium channels is an appropriate treatment protocol for alleviating both insulin resistance and enhancement of insulin secretion in NIDDM patients.

NIDDM pathogenesis is complex and the disease progression occurs in phases. An enhanced β-cell responsiveness provokes and initiates the disease process. It is unclear as to what the actual enhanced activity is and what the triggering mechanisms are for this first phase. It may be an increased secretory response or an increase in β-cell mass. However, there is clearly an enhancement of β-cell activity detected by both basal and postprandial elevated insulin levels denoted as hyperinsulinemia. Consequently, a resulting insulin resistance occurs, phase II, particularly in insulin responsive tissues (muscle, liver, kidney, fat) that function to reduce glucose levels in the blood. A decrease in insulin sensitivity will account for an increase in blood glucose, causing the β-cells to secrete even more insulin to compensate and because of this vicious cycle, full blown NIDDM, marked by an inevitable defect in insulin release, hyperglycemia and insulin

resistance, will characterize the final stage of the disease process.

Each phase of the disease may be characterized by an alteration in $[Ca^{2+}]_i$, and each phase can be treated by a T-type calcium channel antagonist. The electrical β-cell is equipped with two types of voltage-dependent calcium channels, L-type and T-type calcium channels. L-type calcium channels, activated at high voltages, having large unitary conductance, and dihydropyridine-sensitive, are considered the major pipeline for calcium influx into the β-cell (especially at high voltage depolarization). T-type calcium channels, activated at low voltages, with small unitary conductance, and dihydropyridine-insensitive, are important for maintaining basal $[Ca^{2+}]_i$ (Fig. 8), as well as enhancing electrical activity during cell depolarization. T-type calcium channels normally facilitate insulin secretion in β-cells by enhancing cell electrical activity. This modulatory function of T-type calcium channels in insulin secretion is significant during phase I prior to onset of diabetes. Antagonizing these T-type calcium channels will decrease β-cell hyper-responsiveness and consequent hyperinsulinemia arresting the pathogenic pathways that lead to NIDDM.

If hyperinsulinemia and associated insulin resistance has already occurred, a T-type calcium channel blocker is still the appropriate treatment protocol. The insulin responsive tissues, those that are primarily responsible for taking up glucose for re-establishing euglycemia, have elevated basal $[Ca^{2+}]_i$ during hyperinsulinemic conditions. Indeed, it is the elevated basal $[Ca^{2+}]_i$ that precipitates the decrease in insulin sensitivity of these tissues and it is now known that most of these insulin responsive tissues express T-type calcium channels. A T-type calcium channel blocker will

reduce the basal $[Ca^{2+}]_i$ and alleviate the decreased insulin sensitivity.

Once NIDDM has manifested, it is characterized by altered glucose metabolism, a result of abnormal glucose stimulus-secretion responsiveness of β-cells. β-cell desensitization to glucose is the principal secretory defect of NIDDM. L-type and T-type calcium channels, under normal conditions, work in concert promoting the rise in $[Ca^{2+}]_i$ during glucose-stimulated insulin secretion. In NIDDM, this partnership is broken and the necessary rise in $[Ca^{2+}]_i$ for insulin secretion is compromised.

The data herein indicates that L-type calcium channels are finely regulated by basal calcium levels (Figs. 9A-9D). A very small rise in basal calcium will substantially decrease the L-type calcium current and severely reduce the depolarization-induced rise in $[Ca^{2+}]_i$ (Figs. 10 and 11). The data herein also suggests that T-type calcium channels are a primary regulator of resting basal $[Ca^{2+}]_i$ in β-cells. Furthermore, the negative feedback regulation of T-type calcium channels by elevated $[Ca^{2+}]_i$ is absent (Figs. 9A-9D). It is under circumstances of enhanced T-type calcium current activity as seen in the GK rat model of NIDDM and in the neonate streptozotocin-induced diabetes model, that basal $[Ca^{2+}]_i$ is elevated, and a defect in the glucose-stimulated insulin secretion is observed. Simply reducing the basal calcium influx by pharmacological intervention, in situations of enhanced T-type calcium channel expression, may reduce basal $[Ca^{2+}]_i$ in β-cells (Fig. 8) and alleviate the $[Ca^{2+}]_i$-induced inhibition of L-type calcium channels.

There is a clear link between $[Ca^{2+}]_i$ and diabetes. A primary abnormality in $[Ca^{2+}]_i$ handling by cells is the defect initiating parallel impairments in insulin

secretion and insulin action, as well as initiating diabetic complications. Consequent metabolic derangements may further aggravate alterations in $[Ca^{2+}]_i$ homeostasis, creating a relentless cycle leading to progressive deterioration in the overall health of the diabetic patient. Pharmacological agents that regulate $[Ca^{2+}]_i$ homeostasis are thus appropriate therapeutic measures. The use of T-type calcium channel blockers will thus effectively treat and perhaps cure diabetes mellitus.

EXAMPLE III

Pharmacology of Mibefradil Action

It has been shown that mibefradil has a potent inhibitory effect on T-type Ca^{2+} current in vascular smooth muscle cells. The data herein demonstrates that, in convention whole cell patch clamp configuration, mibefradil also blocks T-type Ca^{2+} current in pancreatic β-cells. Mibefradil (1 μM) had been administered in the recording chamber at time zero (Fig. 13), the control (no drug) showed "run down". This figure shows that T-type Ca^{2+} current is more sensitive to mibefradil than the L-type Ca^{2+} current in pancreatic β-cells.

The blockade of T-type Ca^{2+} channels in β-cells with mibefradil is reversible. Fig. 14 demonstrates the reversibility of blockade of T-type Ca^{2+} currents by mibefradil. In these experiments, a very little volume of mibefradil or $NiCl_2$ was delivered near the recording cell. The drug then diffused away from the cell. The final concentration in the chamber was 1 nM. This experiment shows the inhibitory effect of mibefradil on T-type Ca^{2+} current in pancreatic β-cells results from reversible interaction between the drug and the channel protein.

In β-cells, T-type Ca^{2+} channels could mediate a small, but sustained, Ca^{2+} influx by means of their unique "window" current at voltages near resting membrane potentials. Like other voltage-regulated channels, T-type Ca^{2+} channels are opened and closed depending upon the potentials across the cell membranes. This voltage dependency is illustrated in Fig. 15. The activation and inactivation curves represent the percentage of the channels in either open or closed states over a range of voltages. Unlike most of the voltage-dependent Na^{+} channels or L-type Ca^{2+} channels, the activation and inactivation curves of T-type Ca^{2+} channels overlap at the certain range of low voltages (i.e. window). In other words, there is a small portion of T-type Ca^{2+} channels that stay in non-inactivated states in this voltage range. The data in Fig. 15 was obtained from experiments conducted under 10 mM external Ca^{2+} condition, which shifted the window current about 10 mV toward positive voltage due to the surface charge effects of divalent ions on the channels.

The existence of a window current provides a negative feedback regulation of $[Ca^{2+}]_i$ in β-cells. When cells are under an unhealthy condition, they may be slightly depolarized to activate window current, which elevates the basal $[Ca^{2+}]_i$ to protect the cells from further Ca^{2+} influx through the L-type Ca^{2+} channels. This process is reversible if the membrane potential is reset to the normal resting potential (-70 mV).

Mibefradil regulates basal $[Ca^{2+}]_i$ in pancreatic β-cells:

The data herein demonstrates the roles of T-type calcium currents in modulating basal $[Ca^{2+}]_i$ in INS-1 cells (Fig. 8). $[Ca^{2+}]_i$ was directly measured by the ratio of fluorescence excitations at Ca^{2+}-bound (380 nm)

to unbound (340 nm), and then the ratio was converted to the calcium concentration. The bath solution contained 10 mM NaCl, 4 mM KCl, 2 mM $CaCl_2$, and 2 mM $MgCl_2$. In a single cell exhibiting fluctuating basal $[Ca^{2+}]_i$ with an average value near 150 nM, administering 1 μM mibefradil into the chamber immediately lowered the basal calcium. This data shows the T-type calcium currents participate in regulating the mean basal $[Ca^{2+}]_i$ in cultured β-cells.

Mibefradil regulates basal insulin secretion:

The activation of T-type Ca^{2+} channel at low voltage near the resting membrane potential of pancreatic β-cells suggests that the channels are responsible for the Ca^{2+} influx required for insulin secretion under non-stimulus conditions. The NIT-1 cell line was chosen to demonstrate the effect of mibefradil on the basal insulin secretion. NIT-1 is a cell line derived from the β-cell of non-obese-diabetic mouse. This cell line expressed high levels of T-type Ca^{2+} current. The data herein shows that 5 μM mibefradil reduced the basal insulin secretion to less than 40% of control (Fig. 17), indicating this drug is able to lower the high basal insulin secretion level seen during the earlier stage of NIDDM.

Spontaneous elevation of $[Ca^{2+}]_i$:

To demonstrate that T-type Ca^{2+} channels play an important role in calcium entry under non-stimulatory conditions, and therefore regulate basal $[Ca^{2+}]_i$, spontaneous elevations of intracellular free calcium concentration was detected with the Fluo-3 AM fluorescent imaging method. NIT-1 cells were cultured in medium containing 3.3 mM glucose and preloaded with 2.5 μM Fluo-3 AM. The numbers of spontaneous calcium elevated cells were counted and compared to the total cells being used

for a 10 minute observation period. 10 μM $NiCl_2$ inhibited 90% of spontaneous elevation of basal Ca^{2+}.

The cellular mechanism of the spontaneous elevation of intracellular Ca^{2+} was investigated with the epi-fluorescence measurement method. Some INS-1 cells were observed to exhibit transient spontaneous elevations of $[Ca^{2+}]_i$, "Calcium spikes", under non-stimulatory conditions. The Role of T-type Ca^{2+} channels in this spontaneous process was examined as well. In a single cell with spontaneous calcium spike activity (Fig. 17), $NiCl_2$ (30 μM) reduced the frequency of spontaneous calcium spikes immediately. This result suggests that either the T-type Ca^{2+} channels alone or together with the L-type Ca^{2+} channels are responsible for the transient spontaneous elevation of $[Ca^{2+}]_i$, under conditions where no glucose is present. These spontaneous calcium spikes may contribute to basal insulin secretion and control of basal $[Ca^{2+}]_i$.

However, neither mibefradil nor $NiCl_2$ exhibited their effect on basal $[Ca^{2+}]_i$ in all of the β-cells. It was observed that only those cells which had relatively higher initial basal $[Ca^{2+}]_i$ will respond to the T-type Ca^{2+} channel antagonists (Fig. 18). Whereas those cells with lower initial basal $[Ca^{2+}]_i$ had no or less response to the T type Ca^{2+} channel antagonists. This result indicates that T type Ca^{2+} channel antagonists may selectively act on the cells with high basal $[Ca^{2+}]_i$ and bring it back to normal, by inhibiting the window current.

EXAMPLE IV

Action on Pancreatic β-cells

T type Ca^{2+} may play two pathological roles in NIDDM. At the earlier stage, the NIDDM patients exhibit

hyperinsulinemia and β-cell hyperexcitability. This may, at least in part, be due to increased activity of T type Ca^{2+} channel in β-cells. At the more developed NIDDM stage, over-expressed T type Ca^{2+} channel and membrane depolarization resulted from reduced generation of ATP, and may set up a window current in β-cells that causes chronic elevation of basal Ca^{2+} in the β-cells. The elevated basal Ca^{2+} will reduce the L-type Ca^{2+} activity and glucose induced insulin secretion.

It has been shown that mibefradil prevented and reversed development of hyperinsulinemia in rat. This result indicates this drug is a valuable candidate for the treatment of earlier stage NIDDM or for preventing NIDDM in the potential patients.

A series of experiments were conducted with INS-1 cells to show that T type Ca^{2+} facilitated insulin secretion by enhancing the general excitability of pancreatic β-cells. Particularly, activation of T type Ca^{2+} channels will increase the firing frequency of the depolarizing spikes mediated by opening L type Ca^{2+} channels (Fig. 19A). Activation of T type Ca^{2+} channel will also decrease the time of developing action potential elicited by up-threshold depolarizations (Fig. 19B).

To further establish that T type Ca^{2+} current enhances β-cell excitability, 100 μM $NiCl_2$ was administered to effectively block T type Ca^{2+} channels. In contrast to control experiments, $NiCl_2$ caused a delay in the onset of an action potential and a decrease in number of action potentials.

To directly demonstrate the role of T type Ca^{2+} current in glucose-induced insulin secretion, INS-1 cells were incubated with 11.1 mM glucose and variable concentrations of $NiCl_2$, and insulin release was measured.

$NiCl_2$ reduced insulin secretion in a dose-dependent manner (Fig. 20A). On the other hand, clonal insulin secreting cells (HIT-T15, which did not consistently exhibit T type Ca^{2+} current) were not affected by $NiCl_2$ (Fig. 20B).

These results show that T type Ca^{2+} channels play an important role in β-cell excitability and antagonists of T type Ca^{2+} channels (such an $NiCl_2$) will effectively reduce the excitability of β-cells.

Although T type Ca^{2+} channels facilitate insulin secretion by enhancing general excitability of β-cells, the function of T type Ca^{2+} channels is a doubled-edged sword. Under the condition of over-expressed T type Ca^{2+} channel in β-cells, the function of the window current will become dominant and result in an elevation of basal Ca^{2+}. High $[Ca^{2+}]_i$ may cause impairment of insulin release by inactivating L type Ca^{2+} channels.

L-type Ca^{2+} channels are partially inactivated by $[Ca^{2+}]_i$ in non-stimulus condition in β-cells:

Upon establishment of a whole-cell patch, within the first five minutes, the L type Ca^{2+} current "runs-up", as the magnitude of the peak current increases over time in INS-1 cells (Fig. 21). This phenomenon is a universal feature in these cells under the recording conditions used. The pipette solutions contained no ATP but did contain high concentrations of the calcium chelating agents BAPTA and EGTA. When the pipette solution contained high Ca^{2+}, this run-up does not occur. Instead, a rapid run down occurs. The "run-up" phenomenon is likely due to calcium chelation inside the cells. T type Ca^{2+} currents do not show this effect.

Intracellular perfusion patch clamp experiments demonstrated that basal $[Ca^{2+}]_i$ regulates L type Ca^{2+} current amplitude in INS-1 cells:

Intracellular perfusion of a solution containing high Ca^{2+} (Fig. 9A) causes a substantial reduction in the L type Ca^{2+} current. L type Ca^{2+} currents were elicited by a voltage step to +10 mV from a holding potential of -80 mV. The $[Ca^{2+}]_i$ was measured directly using fura-2 ratiometric fluorescence. The effect of a high $[Ca^{2+}]_i$ (272 nM) on the IV relationship is shown in Fig. 9B. Perfusing in high $[Ca^{2+}]_i$, substantially reduces the high voltage current component, but does not affect the low current component. The high $[Ca^{2+}]_i$ caused a shift in peak current to negative voltages, and Ca^{2+} currents were enhanced at negative voltages. This effect seemed to result in a potentiation of the T type Ca^{2+} current (Fig. 9D). Slow deactivating T type Ca^{2+} currents showed a shift in activation upon perfusion of high $[Ca^{2+}]_i$. This may account for the shift in IV. Various concentrations of $[Ca^{2+}]_i$ regulated the activity of L type Ca^{2+} channels (Fig. 9C). Perfusing a low $[Ca^{2+}]_i$ from an existing high $[Ca^{2+}]_i$ (632 nM to 0 nM) caused an increase in the L type Ca^{2+} current over time, however perfusing in high $[Ca^{2+}]_i$ (0 nM to 272 nM and 0 nM to 632 nM) inhibits the L type Ca^{2+} current over time. The levels of $[Ca^{2+}]_i$ therefore have regulatory effects on both the L type Ca^{2+} current and T type Ca^{2+} current, with $[Ca^{2+}]_i$ having significant feedback regulation on the L type Ca^{2+} current.

Effect of basal $[Ca^{2+}]_i$ on Ca^{2+} influx:

The effect of basal $[Ca^{2+}]_i$ on Ca^{2+} influx was examined using the Ca^{2+} dye indicator fura-2 and fluorescence measurements. Voltage-dependent Ca^{2+} influx in a single cell was obtained by perfusion of an

osmotically balanced solution containing 50 mM KCl into the recording chamber. Voltage-dependent increases in $[Ca^{2+}]_i$ occur primarily through nifedipine sensitive Ca^{2+} channels. The resting basal $[Ca^{2+}]_i$ in INS-1 cells was approximately 60-80 nM under the experimental conditions. $[Ca^{2+}]_i$ was determined by a standard curve obtained from a fura-2 calcium imaging kit (Molecule Probes). The empirical K_d obtained for calcium binding to fura-2 in the system was 296 ± 20 nM. When basal $[Ca^{2+}]_i$ remains low, subsequent voltage stimulation with 50 KCl induces rapid and large calcium influx into the cell and these calcium changes are stereotyped upon repetitive stimulation when basal calcium is restored (Fig. 10). In this experiment, following the 50 KCl depolarization, the cell was repolarized by perfusion of the original 5 mM KCl solution. After repolarization, basal $[Ca^{2+}]_i$ slowly reset and then a second 50 KCl depolarization induced a similar $[Ca^{2+}]_i$ transient. When the basal calcium is not allowed to reset, a defect in the second voltage induced calcium transient occurs (Fig. 11). In this experiment, after repolarization, the second depolarization was applied before basal $[Ca^{2+}]_i$ could return to its original value, and thus, the $[Ca^{2+}]_i$ transient is substantially reduced. These findings suggest that basal $[Ca^{2+}]_i$ plays a prominent role in the regulation of voltage dependent Ca^{2+} influx in INS-1 cells. Therefore effectors of basal $[Ca^{2+}]_i$ will have important impact on the amount of calcium that can enter the cell.

Streptozotocin induced high basal $[Ca^{2+}]_i$ inhibits KCl stimulated Ca^{2+} influx:

To reiterate the importance of basal $[Ca^{2+}]_i$ on voltage stimulated Ca^{2+} influx, basal $[Ca^{2+}]_i$ in INS-1 cells was artificially enhanced by pretreating the cells

with the toxicant, streptozotocin. Though it is know that streptozotocin induces DNA strand breaks, it has also been shown to induce Ca^{2+} channel activity in β-cells. The data shows that pretreating cells with 5 mM streptozotocin for 1 hour, followed by 3 hour recovery, causes a two-fold increase in basal calcium (Fig. 22). These cells when stimulated by 50 KCl had reduced calcium influx compared to control cells.

EXAMPLE V

Inhibition of T type Calcium Channel with Mibefradil Metabolite

It has been shown that mibefradil (Ro 40-5967) exerts a selective inhibitory effect on T-type Ca^{2+} currents, although at higher concentrations it can antagonize high voltage-activated Ca^{2+} currents. The action of mibefradil on Ca^{2+} channels is use- and steady state-dependent and the binding site of mibefradil on L-type Ca^{2+} channels is different from that of dihydropyridines. By using conventional whole-cell and perforated patch-clamp, mibefradil is shown to have an inhibitory effect on both T- and L-type Ca^{2+} currents in insulin-secreting cells. However, the effect on L-type Ca^{2+} currents was time-dependent and poorly reversible in perforated patch experiments. Using mass spectrometry it was demonstrated that mibefradil was trapped inside cells and furthermore, a metabolite of mibefradil was detected. Intracellular application of this metabolite selectively blocked the L-type Ca^{2+} current whereas mibefradil exerted no effect. This study shows that mibefradil permeates into cells and is hydrolyzed to a metabolite that blocks L-type Ca^{2+} channels specifically by acting at the inner side of the channel.

Whole-cell patch clamp and a bath perfusion system were first used to establish the dose-dependent inhibition of mibefradil on both T- and L-types of Ca^{2+} currents. The T-type Ca^{2+} current was measured at -30 mV when the membrane was held at -90 mV and the L-type current was measured at +20 mV when the membrane was held at -40 mV. The currents were measured twice at each concentration of mibefradil with 2 min in between measurements. The dose dependent inhibition of T-type Ca^{2+} current is shown in Fig. 3A. The 50% inhibitory concentration (IC_{50}) was 865 nM. No time-dependent inhibition was observed. In contrast, the inhibition of L-type Ca^{2+} currents could not be fitted with a one-to-one binding curve (Fig. 3B). Administration of 1 μM mibefradil progressively reduced L-type Ca^{2+} current up to 70% of the beginning amplitude after 10 minutes (n = 4), which indicated that a more complicated pharmacological mechanism was involved in the action of mibefradil on the L-type Ca^{2+} currents.

A drug diffusing system was then used to test the reversibility of the antagonism of T- and L-type Ca^{2+} currents by mibefradil. Small volumes (approximately 2 μl) of drugs were delivered in close proximity to the recording cell with a quartz capillary positioned by a micromanipulator. After administration, drugs diffused throughout the entire recording chamber containing 2 ml of bath solution. This drug diffusing system was used to test the reversibility of 30 μM of $NiCl_2$ on the T-type Ca^{2+} currents (Fig. 4). The amplitude of T-type current was rapidly reduced to 40% and gradually returned to 80% of the initial level within 3 minutes. Using this system, it was found that the inhibition of mibefradil on the T-type Ca^{2+} current was clearly reversible. In contrast,

the inhibition of the L-type Ca^{2+} current was poorly reversible (Fig. 4).

The poor reversibility and time-dependent inhibition of the L-type Ca^{2+} current by mibefradil suggested that this drug may have an accumulation effect over time. This hypothesis was tested by applying a very low dose of mibefradil on cells and recording the L-type Ca^{2+} currents for a long time in the perforated patch-clamp configuration. As shown in Fig. 5A, after 25 minutes of 10 nM mibefradil administration, the relative currents were reduced to 70%, whereas the currents remained unchanged for control patches. Incubation of cells with 10 nM mibefradil for two hours resulted in further reduction of current densities as recorded by perforated patches (Fig. 5B). At a concentration of 10 nM, mibefradil exhibited no long-term effect on the T-type Ca^{2+} current.

To test the hypothesis that mibefradil may permeate through the cell membrane to the cytoplasm and be trapped inside cells, the presence of mibefradil was examined in cells pre-incubated with 20 μM of mibefradil using mass spectrometry. After 3 washes, mibefradil (peaked at 496 MW) was still detected in cells (Fig. 6B). The concentration of intracellular mibefradil after one minute incubation was 3.18 ± 0.78 μM ($n = 3$). The localization of mibefradil in cells was examined by measuring the concentration of mibefradil in the pellets and supernatants after lysis of the cells. Most of the mibefradil (92%) was detected in the supernatants and 0% was found in the pellets after washing cells with methanol, indicating that mibefradil was trapped in the cytoplasm. In addition, a peak (MW = 423) was detected which represented a hydrolyzed metabolite of mibefradil, Des-methoxyacetyl mibefradil (dm-mibefradil), which is a

major metabolite as documented previously (Wiltshire et al. 1992). By varying the time of pre-incubation, it was found that dm-mibefradil accumulated inside the cells in a time-dependent manner (Fig. 6A). This accumulation is consistent with the concept that dm-mibefradil has lower membrane permeability than its precursor mibefradil.

It was then tested whether or not mibefradil or dm-mibefradil inhibits L- or T-type Ca^{2+} currents from inside of cells. Both L- and T-type currents were measured in the whole-cell patch clamp configuration when 1 μM of mibefradil or dm-mibefradil was included in the pipette solution. As shown in Figs. 7A and 7B, intracellular application of 1 μM mibefradil did not have inhibitory effects on either L-type or T-type Ca^{2+} currents, whereas the same concentration of dm-mibefradil specifically blocked the L-type Ca^{2+} current. As the bath solution contained no drug in this series of experiments, the inhibitory effect of dm-mibefradil is considered to be acting on the inside domain of L-type Ca^{2+} channels.

The inhibitory effect of dm-mibefradil on T-type Ca^{2+} currents was similar to the effect of mibefradil when it was applied in the bath solution, suggesting that the methoxyacetyl group of mibefradil does not play a key role in binding to the extracellular receptor site of T-type Ca^{2+} channel protein. However, this methoxyacetyl group is necessary for blocking L-type Ca^{2+} channel from the inside of cells, indicating a modification in the methoxyacetyl group of mibefradil can result in a more selective antagonist of T-type Ca^{2+} channels.

EXAMPLE VI

LVA Ca^{2+} Current Mediates Cytokine-Induced Pancreatic β-cell Death

Insulin-dependent diabetes mellitus is characterized by the selective destruction of pancreatic β-cells. Chronic treatment with cytokines induced a low voltage-activated (LVA) Ca^{2+} current in mouse β-cells. The concomitant increase in the basal cytoplasmic free Ca^{2+} concentration ($[Ca^{2+}]_i$) was associated with DNA fragmentation and cell death. Antagonists of LVA Ca^{2+} channels prevented this elevation of basal $[Ca^{2+}]_i$ and DNA fragmentation, and reduced the percentage of cell death. Exposure to cytokines did not affect the profile of Ca^{2+} currents or basal $[Ca^{2+}]_i$ in glucagon-secreting α-cells. An increased Ca^{2+} signal through LVA Ca^{2+} channels may thus be a key feature in cytokine-induced β-cell destruction.

The effects of chronic cytokine treatment on the voltage-sensitive Ca^{2+} currents in primary cultured mouse islet cells was examined. After treatment with IL-1β (25 U/ml) and IFNγ (300 U/ml) for 6 h, an LVA Ca^{2+} current was induced in these cells (Fig. 23A). This current was present in 48% of cytokine-treated mouse islet cells. No such current was observed when the cells were treated with either IL-1β or IFNγ alone. Experiments were conducted at different times recording LVA Ca^{2+} currents induced by cytokines, and the results indicate that no further increase in current density occurs even after treatment for 48 h. This LVA current has not been observed in non-treated cells. The steady state inactivation curve of the cytokine-induced LVA Ca^{2+} currents displayed a low voltage property (Fig. 23E) similar to the inactivation curve of the LVA currents in NOD mouse islets cells. This current was also blocked by $NiCl_2$ (10 μM; n = 4; Fig. 23F). It has been reported that

low concentration of $NiCl_2$ selectively block LVA current, a profound increase in Ca^{2+} current density was observed over the voltages between -20 and 20 mV. These high voltage-activated Ca^{2+} currents are nifedipine sensitive currents (completely blocked by 10 μM nifedipine), and the increase in this current density is similar to the increased L type Ca^{2+} current density observed after treatment of β-cells with serum from IDDM patients.

As α-cells are more resistant to the toxic effects of cytokines than β-cells, the effects of cytokines on the Ca^{2+} currents in a glucagon-secreting cell line (α-TC1) was also examined. This cell line, like α-cells, is more resistant to the cytotoxic effect of cytokines. Treatment of α-TC1 cells with IL-1β and IFNγ failed to induce LVA Ca^{2+} currents and did not alter the current density (Figs. 23C and 23D). Therefore, the induction of LVA Ca^{2+} currents and increased Ca^{2+} current density observed after chronic treatment with cytokines showed specificity for β-cells.

LVA Ca^{2+} channels are activated at low membrane potentials. This unique feature may allow then to regular $[Ca^{2+}]_i$ under nonstimulatory conditions. Indeed, basal $[Ca^{2+}]_i$ in cytokine-treated cells was approximately 3-fold higher than in nontreated cells (Fig. 24A). This increase in basal $[Ca^{2+}]_i$ was blocked by $NiCl_2$ (10 μM), but not by the L type Ca^{2+} channel antagonist, nifedipine (10 μM). Cytokines failed to increase basal $[Ca^{2+}]_i$ in α-TC1 cells (Fig. 24B). These results suggest that Ca^{2+} influx through LVA Ca^{2+} channels is responsible for the cytokine-induced elevation in basal $[Ca^{2+}]_i$ in β-cells.

It has been shown that cytokines induce apoptosis in human pancreatic islet cells. Apoptosis is also the mode of cell death in the development of IDDM in the NOD mouse and in multiple low dose streptozotocin-induced IDDM in

the mouse, and is involved in β-cell destruction. As a marker of apoptosis, DNA fragmentation has been reported to precede β-cell lysis.

β-TC3 cells, a mouse β-cell line, were used to demonstrate the role of LVA Ca^{2+} channels in cytokine-mediated DNA fragmentation. The LVA Ca^{2+} current density was first examined before and after cytokine treatment. The LVA Ca^{2+} current (at V_m = -30 mM) in β-TC3 cells was increased from 1.86 ± 0.33 (pA/pF; n = 30) to 3.45 ± 0.47 (pA/pF; n = 10) after treatment with cytokines (25 U/ml IL-1β, 100 U/ml IFNγ, and 100 U/ml TNFα) for 25 h. This indicates that the LVA Ca^{2+} current in β-TC3 cells is regulated by cytokines, as seen in mouse islet cells. As shown in Fig. 24, cytokine-induced DNA fragmentation displayed a ladder pattern of oligonucleosomal fragments. The three LVA Ca^{2+} channel blockers, $NiCl_2$, amiloride, and mibefradil, all independently prevented cytokine-induced DNA fragmentation. In contrast, nifedipine had not inhibitory effect on DNA fragmentation induced by cytokines. This experiment has been repeated in β-TC3 cells (n = 2) as well as in NIT-1 cells (n = 3), a β-cell line derived from NOD mice, and the same results were obtained.

The function of LVA Ca^{2+} channels in cytokine-mediated cell death in β-TC3 cells was then examined. Many cells died when the medium contained 25 U/ml IL-1β, 100 U/ml IFNγ, and 100 U/ml TNFα; however, $NiCl_2$ (20 μM) effectively reduced the β-cell killing potency of cytokines in both a time- and dose-dependent manner (Figs. 25A and 25B, respectively). In contrast, nifedipine did not exhibit a protective effect. Similar results were obtained from an experiment conducted in NIT-1 cells with mibefradil, which also reduced β-cell death induced by cytokines. These results demonstrate

that LVA Ca^{2+} channels enhance the vulnerability of β-cells to the cytotoxic effects of cytokines.

Although preferred embodiments have been depicted and described in detail herein, it will be apparent to those skilled in the relevant art that various modifications, additions, substitutions and the like can be made without departing from the spirit of the invention and these are therefore considered to be within the scope of the invention as defined in the claims which follow.

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What Is Claimed Is:

1. An isolated nucleic acid molecule encoding a pancreatic T-type calcium channel.

2. The isolated nucleic acid molecule of claim 1 wherein said nucleic acid is deoxyribonucleic acid.

3. The isolated nucleic acid molecule of claim 2 wherein said deoxyribonucleic acid is cDNA.

4. The isolated nucleic acid molecule of claim 3 wherein said nucleic acid molecule has a nucleotide sequence as shown in SEQ ID NO:1.

5. The isolated nucleic acid molecule of claim 1 wherein said nucleic acid molecule encodes an amino acid sequence as shown in SEQ ID NO:2.

6. The isolated nucleic acid molecule of claim 1 wherein said nucleic acid is ribonucleic acid.

7. The isolated nucleic acid molecule of claim 6 wherein said ribonucleic acid is mRNA.

8. An antisense nucleic acid molecule complementary to at least a portion of the mRNA of claim 7.

9. A cell comprising the antisense nucleic acid molecule of claim 8.

10. An expression vector comprising the antisense nucleic acid molecule of claim 8.

11. The expression vector of claim 10 wherein the expression vector is selected from the group consisting of a plasmid and a virus.

12. A cell comprising the expression vector of claim 10.

13. A method of decreasing expression of a pancreatic T-type calcium channel in a host cell, said method comprising introducing the antisense nucleic acid molecule of claim 8 into the cell, wherein said antisense nucleic acid molecule blocks translation of said mRNA so as to decrease expression of said pancreatic T-type calcium channel in said host cell.

14. A ribozyme having a recognition sequence complementary to a portion of the mRNA of claim 7.

15. A cell comprising the ribozyme of claim 14.

16. An expression vector comprising the ribozyme of claim 14.

17. The expression vector of claim 16 wherein the expression vector is selected from the group consisting of a plasmid and a virus.

18. A cell comprising the expression vector of claim 16.

19. A method of decreasing expression of a pancreatic T-type calcium channel in a host cell, said method comprising introducing the ribozyme of claim 14 into the cell, wherein expression of said ribozyme in

said cell results in decreased expression of said pancreatic T-type calcium channel in said cell.

20. A cell comprising the nucleic acid molecule of claim 1.

21. An expression vector comprising the nucleic acid molecule of claim 1.

22. The expression vector of claim 21 wherein said expression vector is selected from the group consisting of a plasmid and a virus.

23. A cell comprising the expression vector of claim 21.

24. A method of increasing expression of pancreatic T-type calcium channel in a host cell, said method comprising:

introducing the nucleic acid molecule of claim 1 into the cell; and

allowing said cell to express said nucleic acid molecule resulting in the production of pancreatic T-type calcium channel in said cell.

25. A method of screening a substance for the ability of the substance to modify T-type calcium channel function, said method comprising:

introducing the nucleic acid molecule of claim 1 into a host cell;

expressing said pancreatic T-type calcium channel encoded by said nucleic acid molecule in the host cell;

exposing the cell to a substance; and

evaluating the exposed cell to determine if the substance modifies the function of the T-type calcium channel.

26. The method of claim 25 wherein said evaluation comprises monitoring the expression of T-type calcium channel.

27. A method of obtaining DNA encoding a pancreatic T-type calcium channel, said method comprising:

selecting a DNA molecule encoding a pancreatic T-type calcium channel, said DNA molecule having a nucleotide sequence as shown in SEQ ID NO:1;

designing an oligonucleotide probe for a pancreatic T-type calcium channel based on SEQ ID NO:1;

probing a genomic or cDNA library of an organism with the oligonucleotide probe; and

obtaining clones from said library that are recognized by said oligonucleotide probe, so as to obtain DNA encoding a pancreatic T-type calcium channel.

28. A method of obtaining DNA encoding a pancreatic T-type calcium channel, said method comprising:

selecting a DNA molecule encoding a pancreatic T-type calcium channel, said DNA molecule having a nucleotide sequence as shown in SEQ ID NO:1;

designing degenerate oligonucleotide primers based on SEQ ID NO:1; and

utilizing said oligonucleotide primers in a polymerase chain reaction on a DNA sample to identify homologous DNA encoding a pancreatic T-type calcium channel in said sample.

29. An isolated nucleic acid molecule encoding a pancreatic T-type calcium channel, said nucleic acid molecule encoding a first amino acid sequence having at least 90% amino acid identity to a second amino acid sequence, said second amino acid sequence as shown in SEQ ID NO:2.

30. A DNA oligomer capable of hybridizing to the nucleic acid molecule of claim 1.

31. A method of detecting presence of a pancreatic T-type calcium channel in a sample, said method comprising:

contacting a sample with the DNA oligomer of claim 30, wherein said DNA oligomer hybridizes to any of said pancreatic T-type calcium channel present in said sample, forming a complex therewith; and

detecting said complex, thereby detecting presence of a pancreatic T-type calcium channel in said sample.

32. The method of claim 31 wherein said DNA oligomer is labeled with a detectable marker.

33. An isolated pancreatic T-type calcium channel protein.

34. The pancreatic T-type calcium channel protein of claim 33 wherein said pancreatic T-type calcium channel protein is encoded by a nucleotide sequence as shown in SEQ ID NO:1.

35. The pancreatic T-type calcium channel protein of claim 33 wherein said pancreatic T-type calcium

channel protein is encoded by an amino acid sequence as shown in SEQ ID NO:2.

36. An isolated pancreatic T-type calcium channel protein encoded by a first amino acid sequence having at least 90% amino acid identity to a second amino acid sequence, said second amino acid sequence as shown in SEQ ID NO:2.

37. An antibody or fragment thereof specific for the pancreatic T-type calcium channel protein of claim 36.

38. The antibody of claim 37 wherein said antibody comprises a monoclonal antibody.

39. The antibody of claim 37 wherein said antibody comprises a polyclonal antibody.

40. A composition comprising the pancreatic T-type calcium channel protein of claim 36 and a compatible carrier.

41. A method of detecting presence of a pancreatic T-type calcium channel protein in a sample, said method comprising:

contacting a sample with the antibody or fragment thereof of claim 37, wherein said antibody or fragment thereof binds to any of said pancreatic T-type calcium channel protein present in said sample, forming a complex therewith; and

detecting said complex, thereby detecting presence of a pancreatic T-type calcium channel protein in said sample.

42. The method of claim 41 wherein said antibody or fragment thereof is labeled with a detectable marker.

43. A method of modifying insulin secretion by pancreatic beta cells, the method comprising modifying levels of functional T type calcium channels in the pancreatic beta cells.

44. The method of claim 43 wherein modifying levels of functional T type calcium channels comprises modifying T type calcium channel gene expression in the pancreatic beta cells.

45. The method of claim 44 wherein modifying T type calcium channel gene expression comprises exposing the pancreatic beta cells to a compound which modifies T type calcium channel gene expression.

46. The method of claim 45 wherein the compound is an antisense oligonucleotide targeted to the T type calcium channel gene.

47. The method of claim 43 wherein modifying levels of functional T type calcium channel comprises exposing the pancreatic beta cells to an inhibitor of the functional T type calcium channel.

48. The method of claim 43 wherein modifying levels of functional T type calcium channel comprises exposing the pancreatic beta cells to a compound which interferes with membrane T type calcium channel formation.

49. The method of claim 43 wherein the pancreatic beta cells are present in a subject having type II diabetes.

50. A method of treating type II diabetes in a subject, the method comprising administering to the subject an amount of a compound effective to modify levels of functional T type calcium channel in the pancreatic beta cells of the subject.

51. The method of claim 50 wherein the compound modifies levels of functional T type calcium channel by modifying T type calcium channel gene expression.

52. The method of claim 51 wherein modifying T type calcium channel gene expression comprises exposing the pancreatic beta cells to a compound which modifies T type calcium channel gene expression.

53. The method of claim 52 wherein the compound is an antisense oligonucleotide targeted to the T type calcium channel gene.

54. The method of claim 50 wherein the compound is an inhibitor of the functional T type calcium channel.

55. The method of claim 50 wherein the compound interferes with membrane T type calcium channel formation.

56. A method of modifying basal calcium levels in cells, the method comprising modifying levels of functional T type calcium channels in the cells.

57. A method of modifying the action potential of L type calcium channels in cells, the method comprising modifying levels of functional T type calcium channels in the cells.

58. A method of modifying pancreatic beta cell death, the method comprising modifying levels of functional T type calcium channels in the pancreatic beta cells.

59. A method of modifying pancreatic beta cell proliferation, the method comprising modifying levels of functional T type calcium channels in the pancreatic beta cells.

60. A method of modifying calcium influx through L type calcium channels in cells, the method comprising modifying levels of functional T type calcium channels in the cells.

T-TYPE CALCIUM CHANNEL

ABSTRACT OF THE DISCLOSURE

The present invention is directed to isolated nucleic acid molecules encoding pancreatic T-type calcium channels. Expression vectors and host cells comprising the nucleic acid molecules are also provided, as well as methods for increasing or decreasing the expression of pancreatic T-type calcium channel in host cells. The invention further provides a method of screening a substance for the ability of the substance to modify T-type calcium channel function, and a method for isolating other pancreatic T-type calcium channel molecules. DNA oligomers capable of hybridizing to the nucleic acid molecule encoding the pancreatic T-type calcium channel are provided, which can be used to detect pancreatic T-type calcium channel in a sample. An isolated pancreatic T-type calcium channel protein is also provided. Antibodies specific for the protein, and fragments thereof, are provided, as are compositions comprising the protein and a compatible carrier. The subject invention further provides a method of modifying insulin secretion by pancreatic beta cells, a method of treating type II diabetes in a subject, a method of modifying basal calcium levels in cells, a method of modifying the action potential of L type calcium channels in cells, a method of modifying pancreatic beta cell death, a method of modifying pancreatic beta cell proliferation, and a method of modifying calcium influx through L type calcium channels in cells.

Fig. 1A

(1) atgctcccccaccggggtcccccggttgcgtgaggacacctcctctgaggggctccgctcgcccctcttcg
(2) **ATG**ctcccccaccggg-tcccccg-ttgcgtgaggacacctcctctgaggggctccgctcgcccctcttcg

(1) gacccccccggggccccggctggccagagg**ATG**gacgaggaggaggatggagcgggcgccgaggagtcggga
(2) gacccccccggggccccggctggccagaggatggacgaggaggaggatggagcgggcgccgaggagtcggga

(1) cagccccgtagcttcacgcagctcaacgacctgtccggggccgggggccggcaggggccgggg
(2) cagccccgtagcttcacgcagctcaacgacctgtccggggccgggggc-ggcaggggccggg-

Fig. 1B

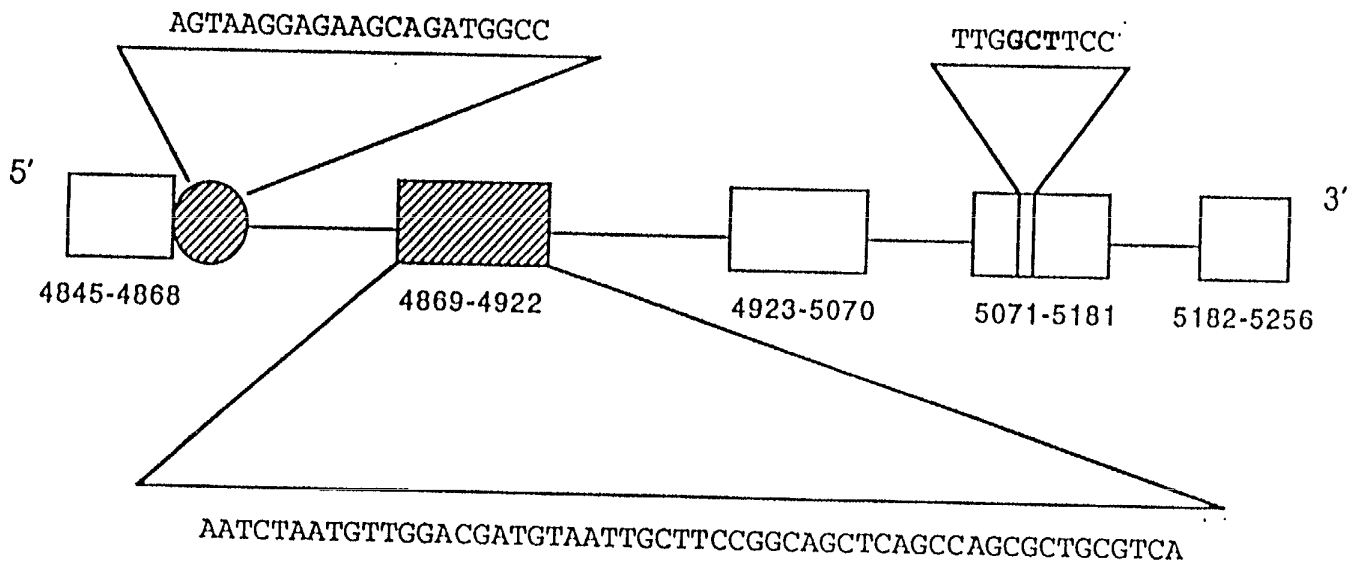


Fig. 2A

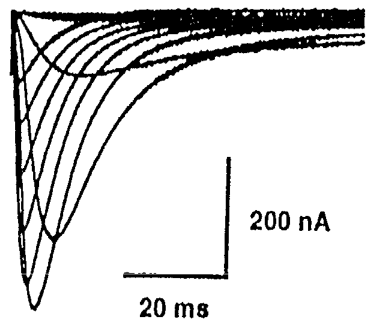


Fig. 2B

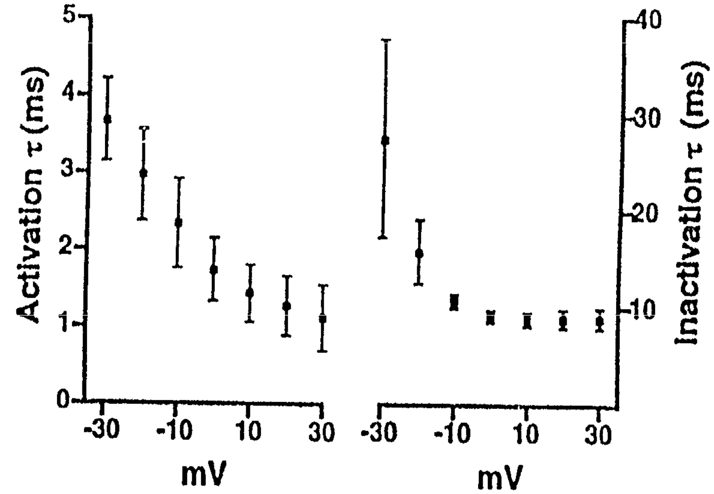


Fig. 2C

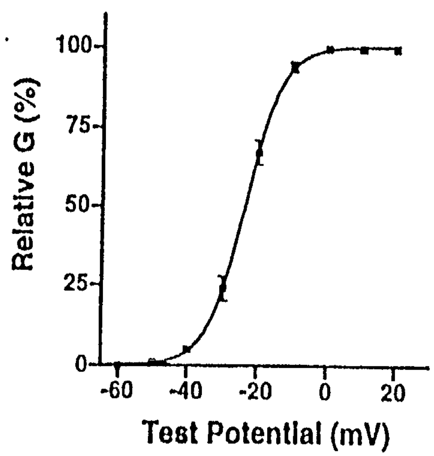


Fig. 2D

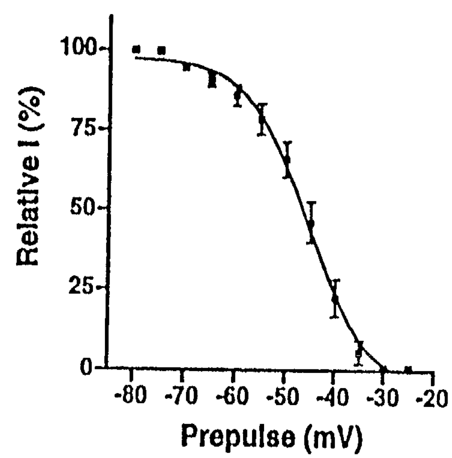
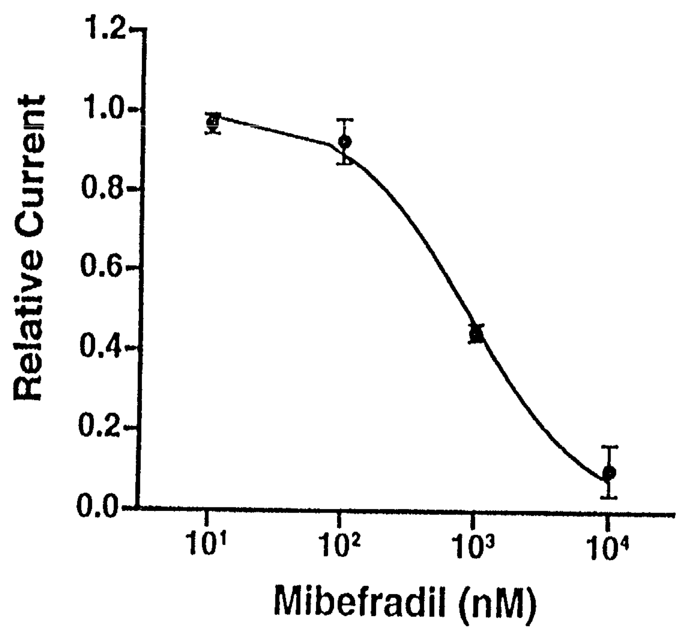


Fig. 3A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0.4
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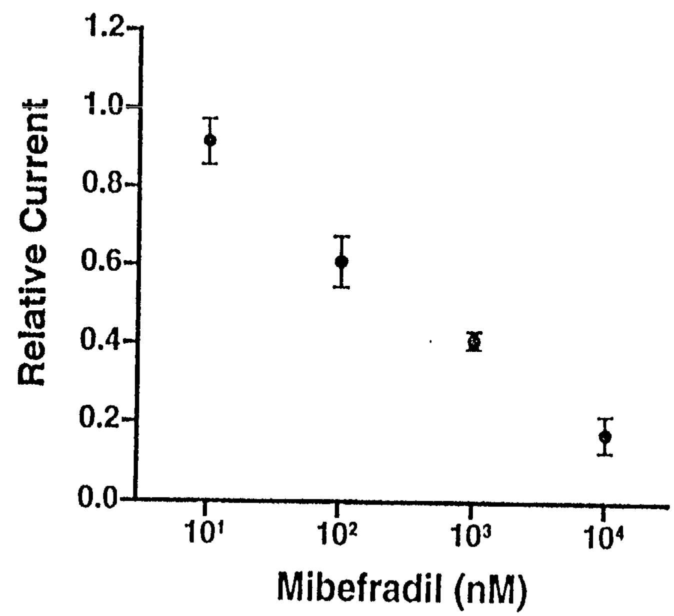
0.0
10^1
10^2
10^3
10^4
Mibefradil (nM)

Fig. 3B


Relative Current
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0.6
0.4
0.2
0.0
10^1
10^2
10^3
10^4
Mibefradil (nM)

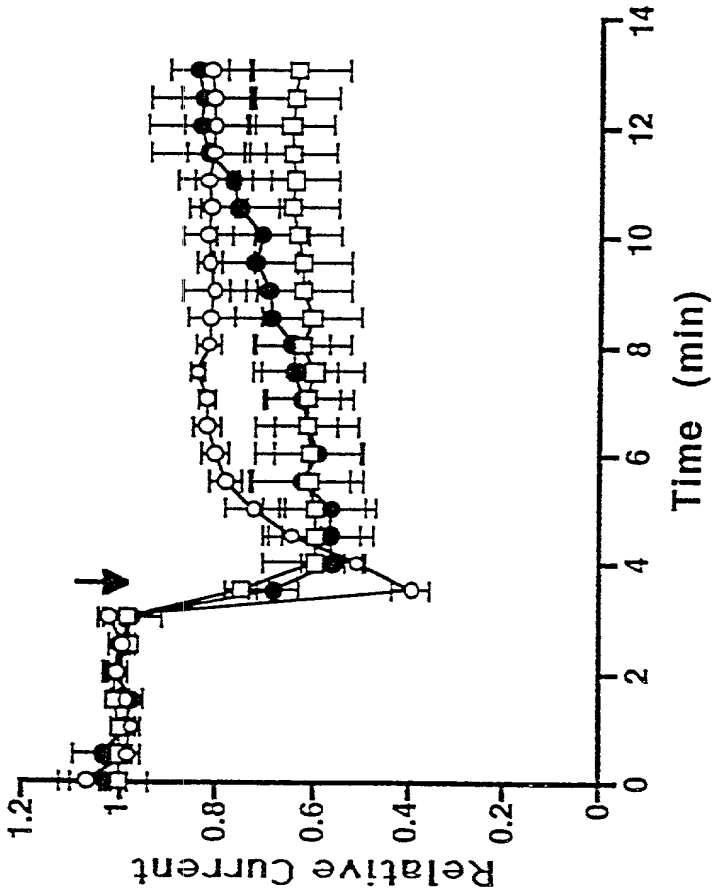
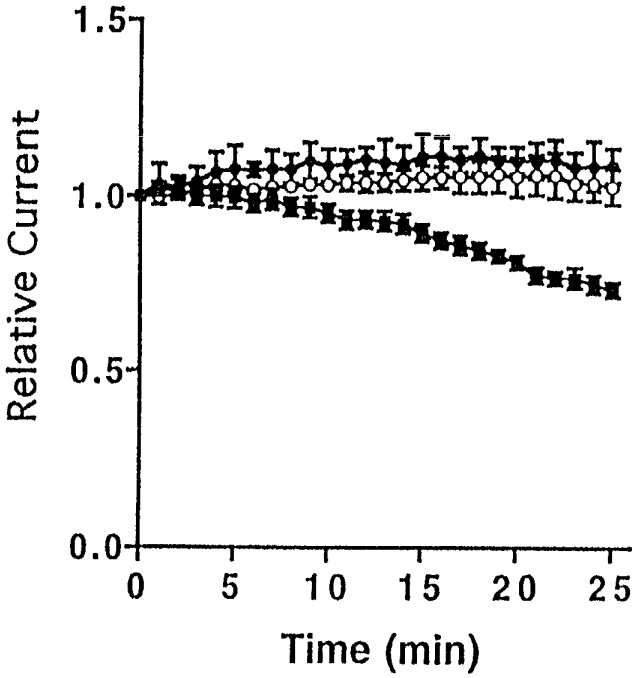


Fig. 4

Fig. 5A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0.5
0.0
Relative Current
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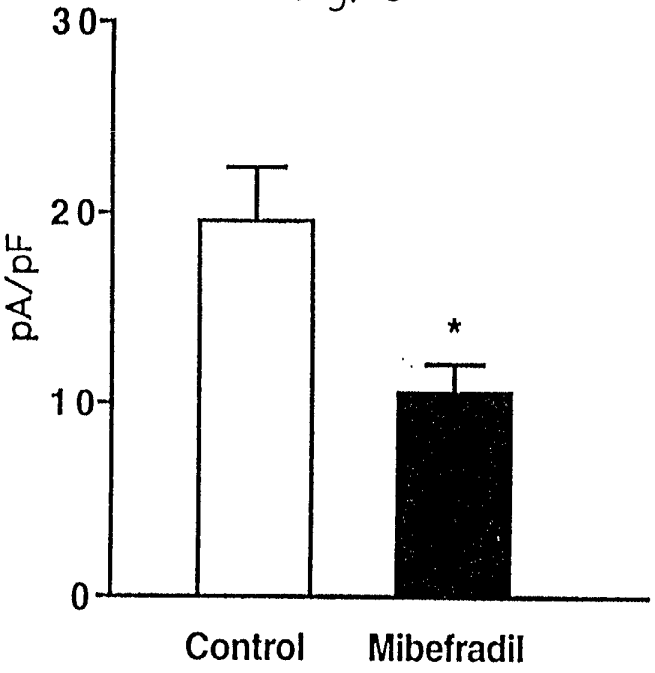
5
10
15
20
25
Time (min)

Fig. 5B


30
20
10
0
pA/pF
*
Control
Mibefradil

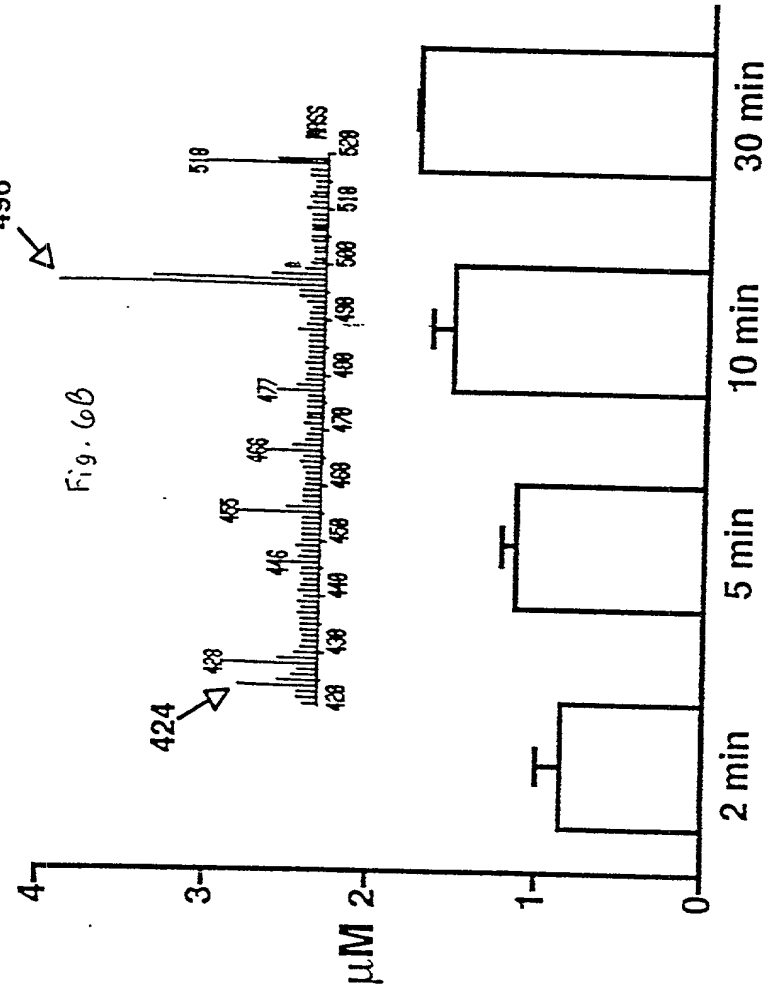


Fig. 6B

Fig. 6A

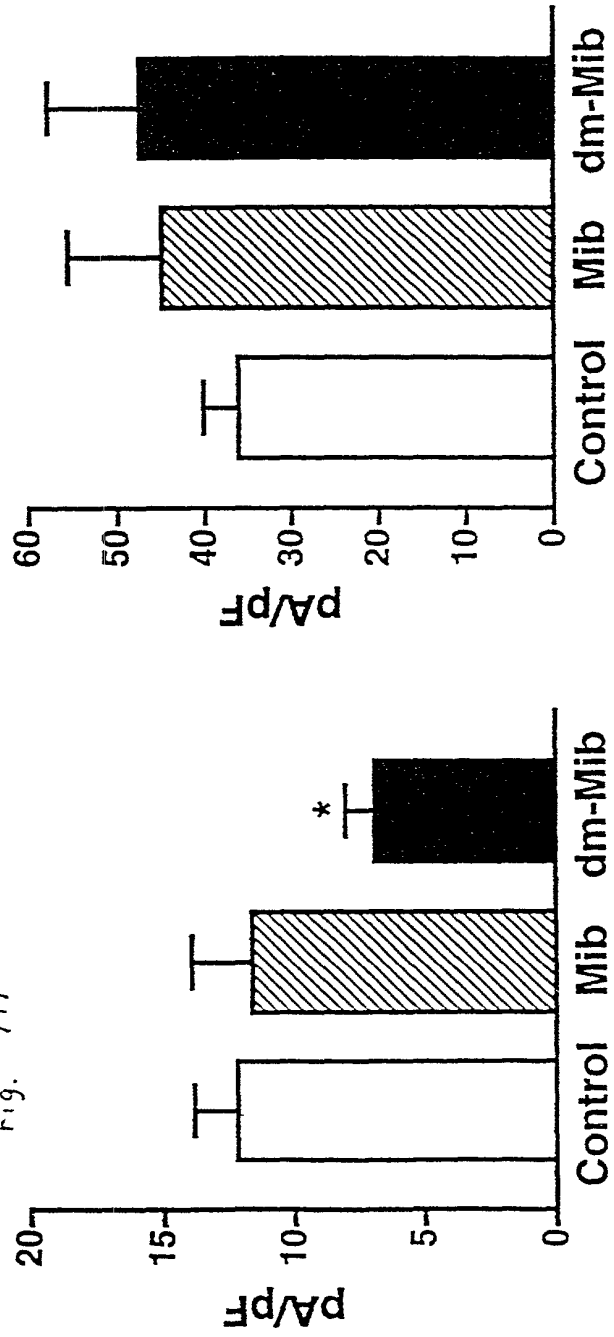

pA/pF
0
5
10
15
20
*
Control
Mib
dm-Mib

Fig. 7A

pA/pF
0
10
20
30
40
50
60
Control
Mib
dm-Mib

Fig. 7B

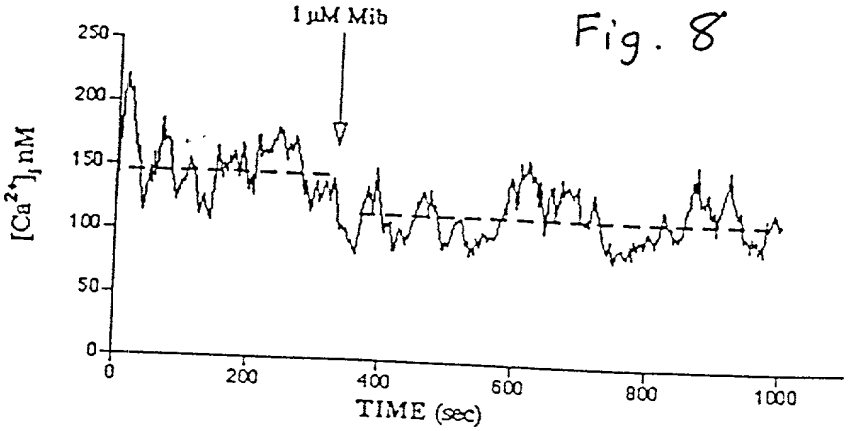


Fig. 8

Fig. 9A

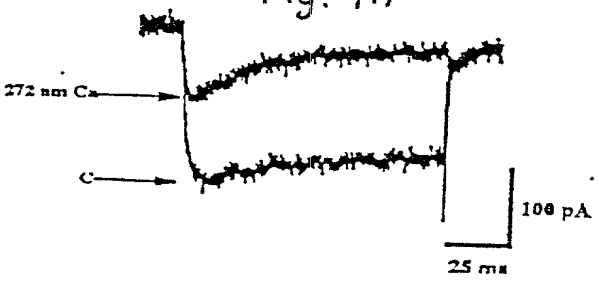
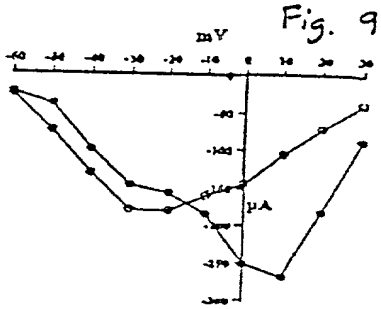


Fig. 9B



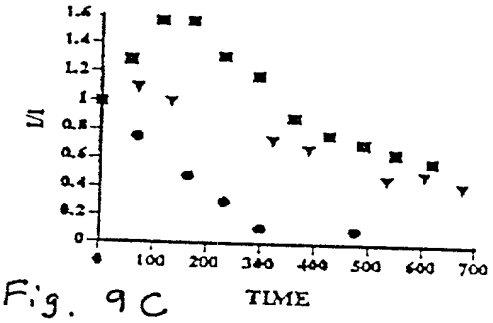


Fig. 9C

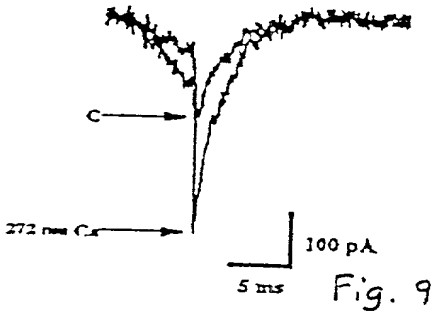


Fig. 9D

Fig. 10

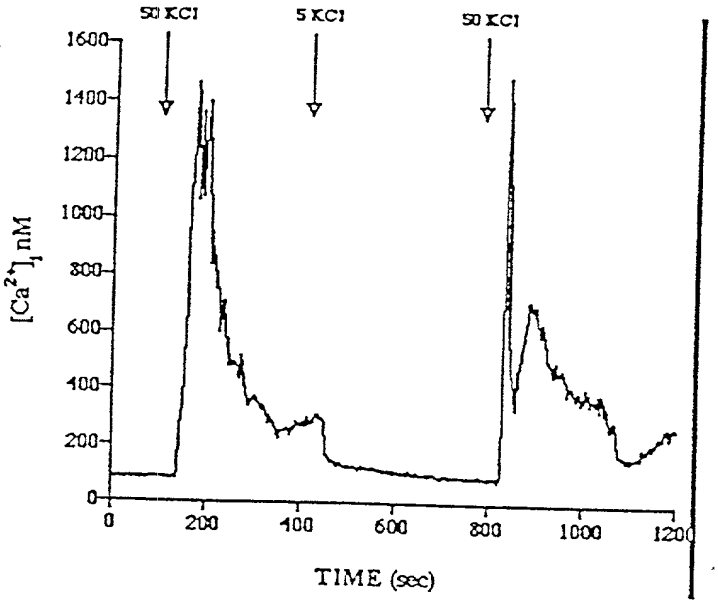

50 KCl
5 KCl
50 KCl
1600
1400
1200
1000
800
600
400
200
0
$[Ca^{2+}]_i$ nM
0
200
400
600
800
1000
1200
TIME (sec)

Fig. 11

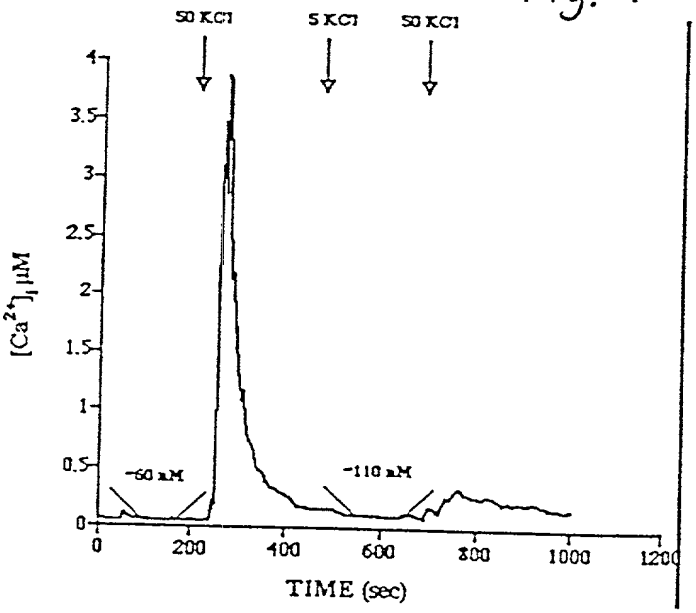

50 KCl
5 KCl
50 KCl
4
3.5
3
2.5
2
1.5
1
0.5
0
$[Ca^{2+}]_i$ µM
~60 nM
~110 nM
0
200
400
600
800
1000
1200
TIME (sec)

Fig. 12

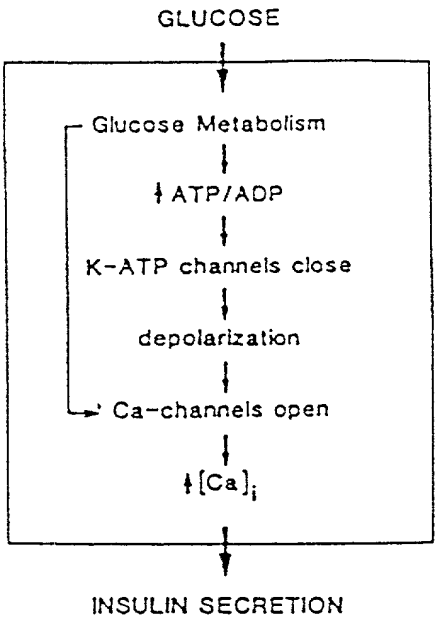
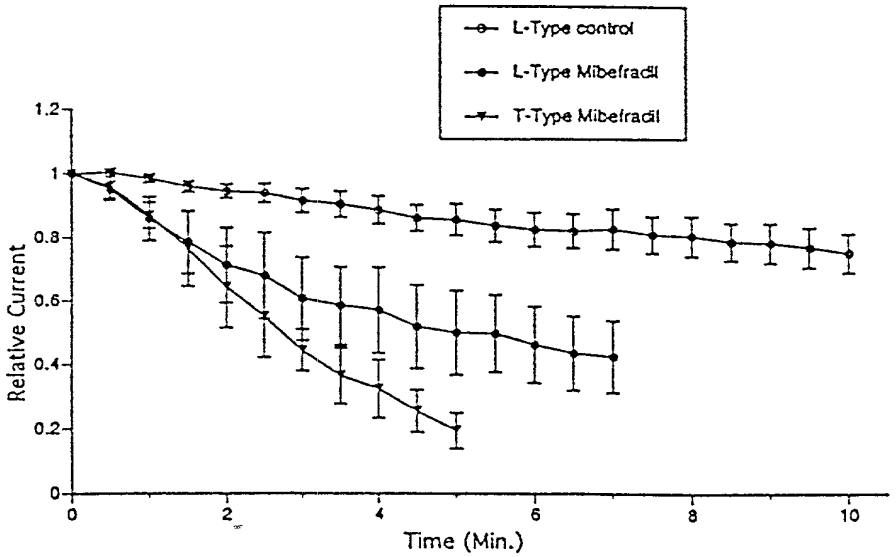

GLUCOSE
Glucose Metabolism
↑ ATP/ADP
K-ATP channels close
depolarization
Ca-channels open
↑ [Ca]i
INSULIN SECRETION

Fig. 13


L-Type control
L-Type Mibefradil
T-Type Mibefradil
Relative Current
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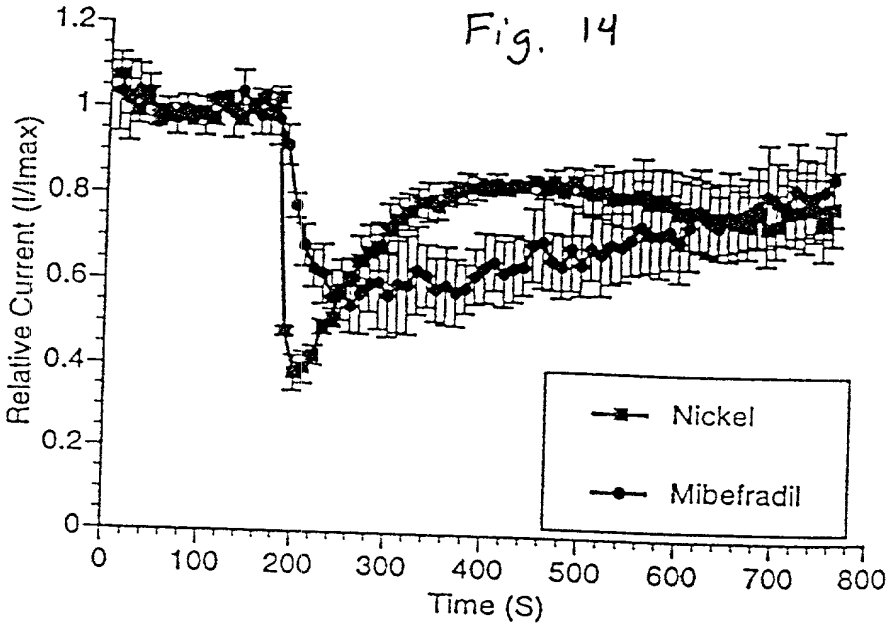
0.6
0.4
0.2
0
0
2
4
6
8
10
Time (Min.)

Fig. 14


Relative Current (I/Imax)
1.2
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0.8
0.6
0.4
0.2
0
0
100
200
300
400
500
600
700
800
Time (S)
Nickel
Mibefradil

Fig. 15

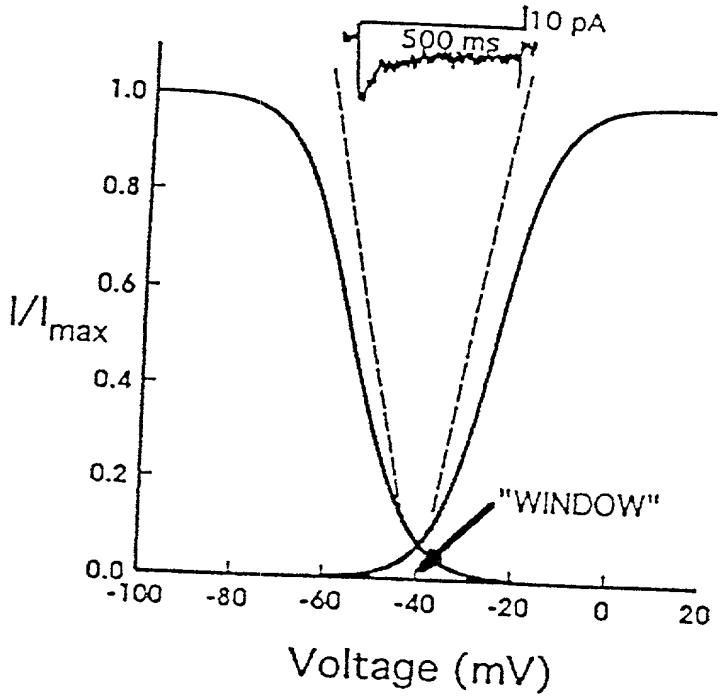
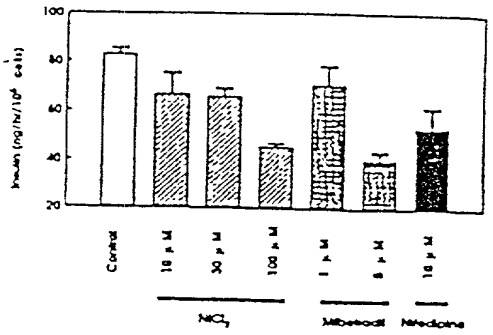

10 pA
500 ms
1.0
0.8
0.6
0.4
0.2
0.0
I/Imax
"WINDOW"
-100
-80
-60
-40
-20
0
20
Voltage (mV)

Fig. 16



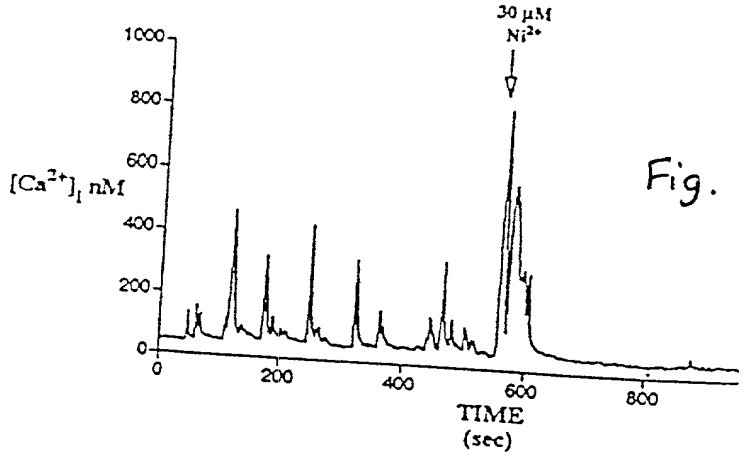
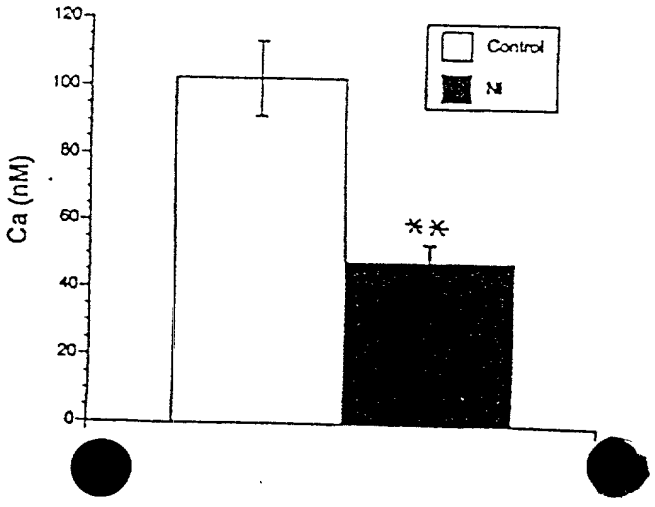


Fig. 17

Fig. 18



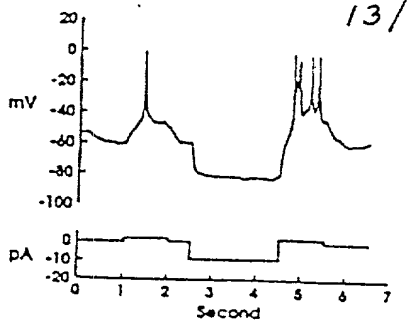


Fig. 19A

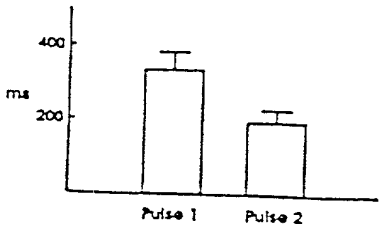


Fig. 19B

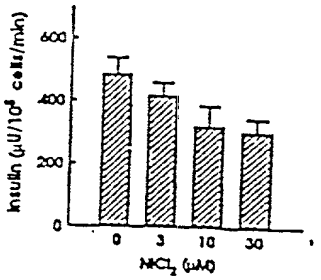


Fig. 20A

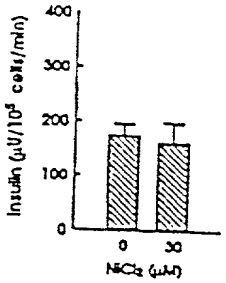
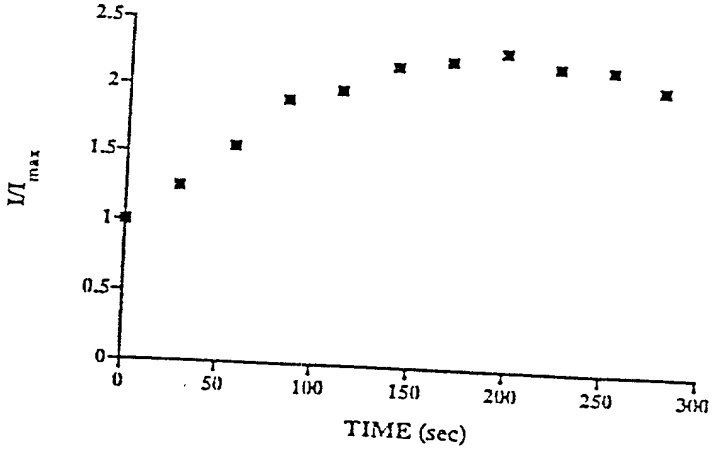


Fig. 20B

Fig. 21



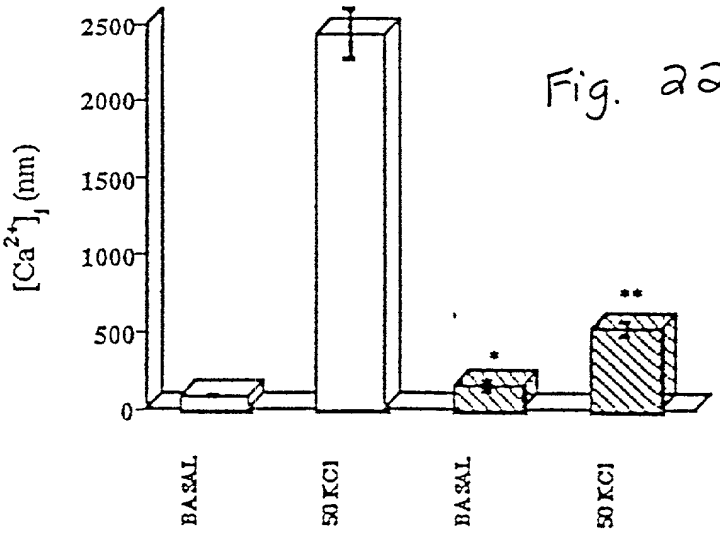


Fig. 22

Fig. 23A.

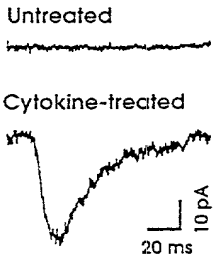


Fig. 23B.

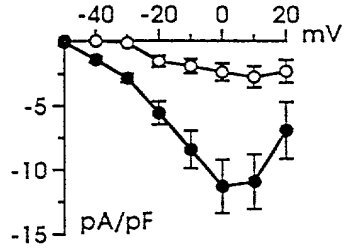


Fig. 23C.

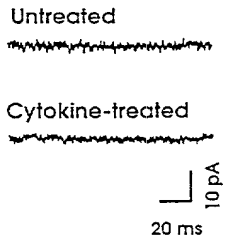


Fig. 23D.

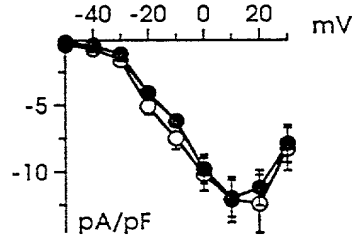


Fig. 23E.

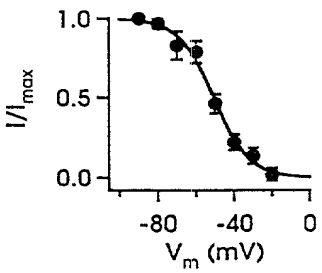


Fig. 23F.

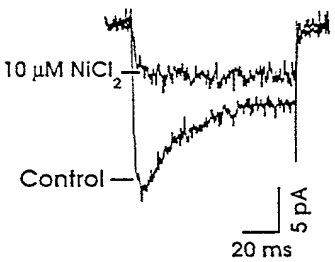


Fig. 24A.

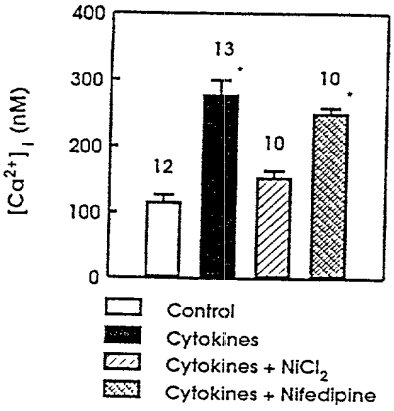


Fig. 24B.

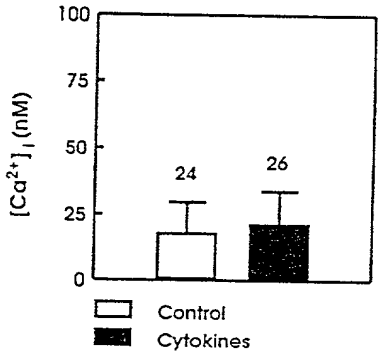


Fig. 25A.

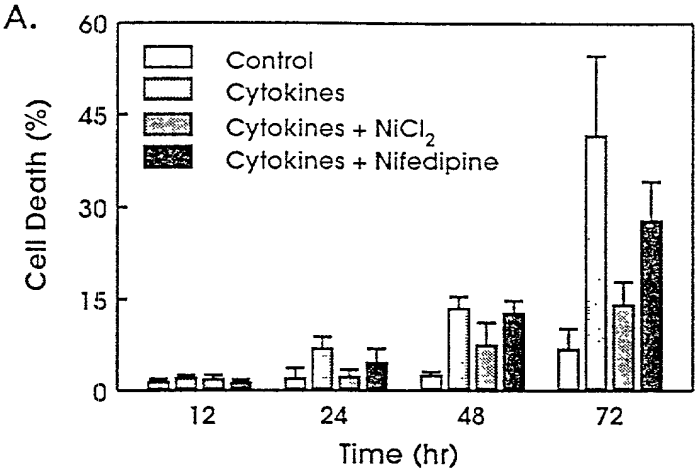
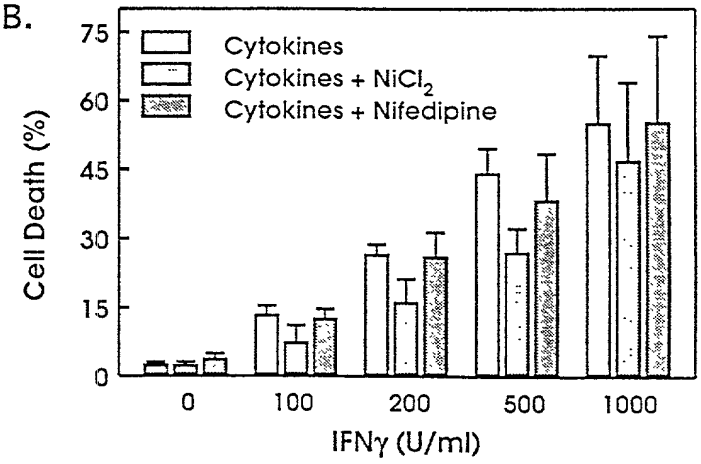


Fig. 25B.



SEQUENCE LISTING

DNASIS DNA Translation [T-INS]
File Name : T-INS
Range : 2 - 7286 Mode : Normal
Codon Table : Universal

start of SEQ ID NO:4

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                10          19          28          37          46          55
5' GAG CTG AGC TGA ACT GGC CCT CCT GGG GAC TCA GCA AGC TCT CTA GAG CCC CCC
---------------------------------------------------------------------------
   E   L   S   *   T   G   P   P   G   D   S   A   S   S   L   E   P   P

                64          73          82          91          100         109
   ACA TGC TCC CCC ACC GGG GTC CCC CGG TTG CGT GAG GAC ACC TCC TCT GAG GGG
   ---------------------------------------------------------------------
    T   C   S   P   T   G   V   P   R   L   R   E   D   T   S   S   E   G

                118         127         136         145         154         163
   CTC CGC TCG CCC CTC TTC GGA CCC CCC GGG GCC CCG GCT GGC CAG AGG ATG GAC
   ---------------------------------------------------------------------
    L   R   S   P   L   F   G   P   P   G   A   P   A   G   Q   R   M   D

                172         181         190         199         208         217
   GAG GAG GAG GAT GGA GCG GGC GCC GAG GAG TCG GGA CAG CCC CGT AGC TTC ACG
   ---------------------------------------------------------------------
    E   E   E   D   G   A   G   A   E   E   S   G   Q   P   R   S   F   T

                226         235         244         253         262         271
   CAG CTC AAC GAC CTG TCC GGG GCC GGG GGC CGG CAG GGG CCG GGG TCG ACG GAA
   ---------------------------------------------------------------------
    Q   L   N   D   L   S   G   A   G   G   R   Q   G   P   G   S   T   E

                280         289         298         307         316         325
   AAG GAC CCG GGC AGC GCG GAC TCC GAG GCG GAG GGG CTG CCG TAC CCG GCG CTA
   ---------------------------------------------------------------------
    K   D   P   G   S   A   D   S   E   A   E   G   L   P   Y   P   A   L

                334         343         352         361         370         379
   GCC CCG GTG GTT TTC TTC TAC TTG AGC CAG GAC AGC CGC CCG CGG AGC TGG TGT
   ---------------------------------------------------------------------
    A   P   V   V   F   F   Y   L   S   Q   D   S   R   P   R   S   W   C

                388         397         406         415         424         433
   CTC CGC ACG GTC TGT AAC CCG TGG TTC GAG CGA GTC AGT ATG CTG GTC ATT CTT
   ---------------------------------------------------------------------
    L   R   T   V   C   N   P   W   F   E   R   V   S   M   L   V   I   L

                442         451         460         469         478         487
   CTC AAC TGT GTG ACT CTG GGT ATG TTC AGG CCG TGT GAG GAC ATT GCC TGT GAC
   ---------------------------------------------------------------------
    L   N   C   V   T   L   G   M   F   R   P   C   E   D   I   A   C   D

                496         505         514         523         532         541
   TCC CAG CGC TGC CGG ATC CTG CAG GCC TTC GAT GAC TTC ATC TTT GCC TTC TTT
   ---------------------------------------------------------------------
    S   Q   R   C   R   I   L   Q   A   F   D   D   F   I   F   A   F   F

                550         559         568         577         586         595
   GCT GTG GAA ATG GTG GTG AAG ATG GTG GCC TTG GGC ATC TTT GGG AAG AAA TGT
   ---------------------------------------------------------------------
    A   V   E   M   V   V   K   M   V   A   L   G   I   F   G   K   K   C

            604         613         622         631         640         649
   TAC CTG GGA GAC ACT TGG AAC CGG CTT GAC TTT TTC ATT GTC ATT GCA GGG ATG
   ---------------------------------------------------------------------
    Y   L   G   D   T   W   N   R   L   D   F   F   I   V   I   A   G   M
```

start of SEQ ID NO:1 & SEQ ID NO:2 (coding regions)

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DNASIS DNA Translation [T-INS]

```
          658        667        676        685        694        703
CTG GAG TAT TCG CTG GAC CTG CAG AAC GTC AGC TTC TCC GCA GTC AGG ACA GTC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 L   E   Y   S   L   D   L   Q   N   V   S   F   S   A   V   R   T   V

          712        721        730        739        748        757
CGT GTG CTG CGA CCG CTC AGG GCC ATT AAC CGG GTG CCC AGC ATG CGC ATT CTC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 R   V   L   R   P   L   R   A   I   N   R   V   P   S   M   R   I   L

          766        775        784        793        802        811
GTC ACA TTA CTG CTG GAC ACC TTG CCT ATG CTG GGC AAC GTC CTG CTG CTC TGT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 V   T   L   L   L   D   T   L   P   M   L   G   N   V   L   L   L   C

          820        829        838        847        856        865
TTC TTC GTC TTT TTC ATC TTT GGC ATC GTG GGC GTC CAG CTG TGG GCA GGA CTG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 F   F   V   F   F   I   F   G   I   V   G   V   Q   L   W   A   G   L

          874        883        892        901        910        919
CTT CGC AAC CGA TGC TTC CTC CCC GAG AAC TTC AGC CTC CCC CTG AGC GTG GAC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 L   R   N   R   C   F   L   P   E   N   F   S   L   P   L   S   V   D

          928        937        946        955        964        973
CTG GAG CCT TAT TAC CAG ACA GAG AAT GAG GAC GAG AGC CCC TTC ATC TGC TCT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 L   E   P   Y   Y   Q   T   E   N   E   D   E   S   P   F   I   C   S

          982        991       1000       1009       1018       1027
CAG CCT CGG GAG AAT GGC ATG AGA TCC TGC AGG AGT GTG CCC ACA CTG CGT GGG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 Q   P   R   E   N   G   M   R   S   C   R   S   V   P   T   L   R   G

         1036       1045       1054       1063       1072       1081
GAA GGC GGT GGT GGC CCA CCC TGC AGT CTG GAC TAT GAG ACC TAT AAC AGT TCC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 E   G   G   G   G   P   P   C   S   L   D   Y   E   T   Y   N   S   S

         1090       1099       1108       1117       1126       1135
AGC AAC ACC ACC TGT GTC AAC TGG AAC CAG TAC TAT ACC AAC TGC TCT GCG GGC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 S   N   T   T   C   V   N   W   N   Q   Y   Y   T   N   C   S   A   G

         1144       1153       1162       1171       1180       1189
GAG CAC AAC CCC TTC AAA GGC GCC ATC AAC TTT GAC AAC ATT GGC TAT GCC TGG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 E   H   N   P   F   K   G   A   I   N   F   D   N   I   G   Y   A   W

         1198       1207       1216       1225       1234       1243
ATC GCC ATC TTC CAG GTC ATC ACA CTG GAG GGC TGG GTC GAC ATC ATG TAC TTC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 I   A   I   F   Q   V   I   T   L   E   G   W   V   D   I   M   Y   F

         1252       1261       1270       1279       1288       1297
GTA ATG GAC GCT CAC TCC TTC TAC AAC TTC ATC TAC TTC ATT CTT CTC ATC ATC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 V   M   D   A   H   S   F   Y   N   F   I   Y   F   I   L   L   I   I

         1306       1315       1324       1333       1342       1351
GTG GGC TCC TTC TTC ATG ATC AAC CTG TGC CTG GTG GTG ATT GCC ACG CAG TTC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 V   G   S   F   F   M   I   N   L   C   L   V   V   I   A   T   Q   F
```

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DNASIS DNA Translation [T-INS]

```
          1360        1369        1378        1387        1396        1405
TCC GAG ACC AAA CAG CGG GAG AGT CAG CTG ATG CGG GAG CAG CGT GTA CGA TTC
---------------------------------------------------------------------
 S   E   T   K   Q   R   E   S   Q   L   M   R   E   Q   R   V   R   F

          1414        1423        1432        1441        1450        1459
CTG TCC AAT GCT AGC ACC CTG GCA AGC TTC TCT GAG CCA GGC AGC TGC TAT GAG
---------------------------------------------------------------------
 L   S   N   A   S   T   L   A   S   F   S   E   P   G   S   C   Y   E

          1468        1477        1486        1495        1504        1513
GAG CTA CTC AAG TAC CTG GTG TAC ATC CTC CGA AAA GCA GCC CGA AGG CTG GCC
---------------------------------------------------------------------
 E   L   L   K   Y   L   V   Y   I   L   R   K   A   A   R   R   L   A

          1522        1531        1540        1549        1558        1567
CAG GTC TCT AGG GCT ATA GGC GTG CGG GCT GGG CTG CTC AGC AGC CCA GTG GCC
---------------------------------------------------------------------
 Q   V   S   R   A   I   G   V   R   A   G   L   L   S   S   P   V   A

          1576        1585        1594        1603        1612        1621
CGT AGT GGG CAG GAG CCC CAG CCC AGT GGC AGC TGC ACT CGC TCA CAC CGT CGT
---------------------------------------------------------------------
 R   S   G   Q   E   P   Q   P   S   G   S   C   T   R   S   H   R   R

          1630        1639        1648        1657        1666        1675
CTG TCT GTC CAC CAC CTG GTC CAC CAC CAT CAC CAC CAC CAT CAC CAC TAC CAC
---------------------------------------------------------------------
 L   S   V   H   H   L   V   H   H   H   H   H   H   H   H   Y   H

          1684        1693        1702        1711        1720        1729
CTG GGT AAT GGG ACG CTC AGA GTT CCC CGG GCC AGC CCA GAG ATC CAG GAC AGG
---------------------------------------------------------------------
 L   G   N   G   T   L   R   V   P   R   A   S   P   E   I   Q   D   R

          1738        1747        1756        1765        1774        1783
GAT GCC AAT GGG TCT CGC CGG CTC ATG CTA CCA CCA CCC TCT ACA CCC ACT CCC
---------------------------------------------------------------------
 D   A   N   G   S   R   R   L   M   L   P   P   P   S   T   P   T   P

          1792        1801        1810        1819        1828        1837
TCT GGG GGC CCT CCG AGG GGT GCG GAG TCT GTA CAC AGC TTC TAC CAT GCT GAC
---------------------------------------------------------------------
 S   G   G   P   P   R   G   A   E   S   V   H   S   F   Y   H   A   D

          1846        1855        1864        1873        1882        1891
TGC CAC TTG GAG CCA GTC CGT TGC CAG GCA CCC CCT CCC AGA TGC CCA TCG GAG
---------------------------------------------------------------------
 C   H   L   E   P   V   R   C   Q   A   P   P   P   R   C   P   S   E

          1900        1909        1918        1927        1936        1945
GCA TCT GGT AGG ACT GTG GGT AGT GGG AAG GTG TAC CCC ACT GTG CAT ACC AGC
---------------------------------------------------------------------
 A   S   G   R   T   V   G   S   G   K   V   Y   P   T   V   H   T   S

          1954        1963        1972        1981        1990        1999
CCT CCA CCA GAG ATA CTG AAG GAT AAA GCA CTA GTG GAG GTG GCC CCC AGC CCT
---------------------------------------------------------------------
 P   P   P   E   I   L   K   D   K   A   L   V   E   V   A   P   S   P

          2008        2017        2026        2035        2044        2053
GGG CCC CCC ACC CTC ACC AGC TTC AAC ATC CCA CCT GGG CCC TTC AGC TCC ATG
---------------------------------------------------------------------
 G   P   P   T   L   T   S   F   N   I   P   P   G   P   F   S   S   M
```

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DNASIS DNA Translation [T-INS]

```
        2062        2071        2080        2089        2098        2107
CAC AAG CTC CTG GAG ACA CAG AGT ACG GGA GCC TGC CAT AGC TCC TGC AAA ATC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 H   K   L   L   E   T   Q   S   T   G   A   C   H   S   S   C   K   I

        2116        2125        2134        2143        2152        2161
TCC AGC CCT TGC TCC AAG GCA GAC AGT GGA GCC TGC GGG CCG GAC AGT TGT CCC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 S   S   P   C   S   K   A   D   S   G   A   C   G   P   D   S   C   P

        2170        2179        2188        2197        2206        2215
TAC TGT GCC CGG ACA GGA GCA GGA GAG CCA GAG TCC GCT GAC CAT GTC ATG CCT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 Y   C   A   R   T   G   A   G   E   P   E   S   A   D   H   V   M   P

        2224        2233        2242        2251        2260        2269
GAC TCA GAC AGC GAG GCT GTG TAT GAG TTC ACA CAG GAC GCT CAG CAC AGT GAC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 D   S   D   S   E   A   V   Y   E   F   T   Q   D   A   Q   H   S   D

        2278        2287        2296        2305        2314        2323
CTC CGG GAT CCC CAC AGC CGG CGG CGA CAG CGG AGC CTG GGC CCA GAT GCA GAG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 L   R   D   P   H   S   R   R   R   Q   R   S   L   G   P   D   A   E

        2332        2341        2350        2359        2368        2377
CCT AGT TCT GTG CTG GCT TTC TGG AGG CTG ATC TGT GAC ACA TTC CGG AAG ATC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 P   S   S   V   L   A   F   W   R   L   I   C   D   T   F   R   K   I

        2386        2395        2404        2413        2422        2431
GTA GAT AGC AAA TAC TTT GGC CGG GGA ATC ATG ATC GCC ATC CTG GTC AAT ACA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 V   D   S   K   Y   F   G   R   G   I   M   I   A   I   L   V   N   T

        2440        2449        2458        2467        2476        2485
CTC AGC ATG GGC ATC GAG TAC CAC GAG CAG CCC GAG GAG CTC ACC AAC GCC CTG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 L   S   M   G   I   E   Y   H   E   Q   P   E   E   L   T   N   A   L

        2494        2503        2512        2521        2530        2539
GAA ATC AGC AAC ATC GTC TTC ACC AGC CTC TTC GCC TTG GAG ATG CTG CTG AAA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 E   I   S   N   I   V   F   T   S   L   F   A   L   E   M   L   L   K

        2548        2557        2566        2575        2584        2593
CTG CTT GTC TAC GGT CCC TTT GGC TAC ATT AAG AAT CCC TAC AAC ATC TTT GAT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 L   L   V   Y   G   P   F   G   Y   I   K   N   P   Y   N   I   F   D

        2602        2611        2620        2629        2638        2647
GGT GTC ATT GTG GTC ATC AGT GTG TGG GAG ATT GTG GGC CAG CAG GGA GGT GGC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 G   V   I   V   V   I   S   V   W   E   I   V   G   Q   Q   G   G   G

        2656        2665        2674        2683        2692        2701
CTG TCG GTG CTG CGG ACC TTC CGC CTG ATG CGG GTG CTG AAG CTG GTG CGC TTC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 L   S   V   L   R   T   F   R   L   M   R   V   L   K   L   V   R   F

        2710        2719        2728        2737        2746        2755
CTG CCG GCC CTG CAG CGC CAG CTC GTG GTG CTC ATG AAG ACC ATG GAC AAC GTG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 L   P   A   L   Q   R   Q   L   V   V   L   M   K   T   M   D   N   V
```

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DNASIS DNA Translation [T-INS]

```
        2764        2773        2782        2791        2800        2809
GCC ACC TTC TGC ATG CTC CTC ATG CTG TTC ATC TTC ATC TTC AGC ATC CTG GGC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 A   T   F   C   M   L   L   M   L   F   I   F   I   F   S   I   L   G

        2818        2827        2836        2845        2854        2863
ATG CAT CTC TTT GGT TGC AAG TTC GCA TCT GAA CGG GAT GGG GAC ACG TTG CCA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 M   H   L   F   G   C   K   F   A   S   E   R   D   G   D   T   L   P

        2872        2881        2890        2899        2908        2917
GAC CGG AAG AAT TTC GAC TCC CTG CTC TGG GCC ATC GTC ACT GTC TTT CAG ATT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 D   R   K   N   F   D   S   L   L   W   A   I   V   T   V   F   Q   I

        2926        2935        2944        2953        2962        2971
CTG ACT CAG GAA GAC TGG AAT AAA GTC CTC TAC AAC GGC ATG GCC TCC ACA TCG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 L   T   Q   E   D   W   N   K   V   L   Y   N   G   M   A   S   T   S

        2980        2989        2998        3007        3016        3025
TCT TGG GCT GCT CTT TAC TTC ATC GCC CTC ATG ACT TTT GGC AAC TAT GTG CTC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 S   W   A   A   L   Y   F   I   A   L   M   T   F   G   N   Y   V   L

        3034        3043        3052        3061        3070        3079
TTT AAC CTG CTG GTG GCC ATT CTT GTG GAA GGA TTC CAG GCA GAG GAA ATC GGC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 F   N   L   L   V   A   I   L   V   E   G   F   Q   A   E   E   I   G

        3088        3097        3106        3115        3124        3133
AAA CGG GAA GAT GCG AGT GGA CAG TTA AGC TGT ATT CAG CTG CCT GTC AAC TCT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 K   R   E   D   A   S   G   Q   L   S   C   I   Q   L   P   V   N   S

        3142        3151        3160        3169        3178        3187
CAG GGG GGA GAT GCC ACC AAG TCT GAG TCA GAG CCT GAT TTC TTT TCG CCC AGT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 Q   G   G   D   A   T   K   S   E   S   E   P   D   F   F   S   P   S

        3196        3205        3214        3223        3232        3241
GTG GAT GGT GAT GGG GAC AGA AAG AAG CGC TTG GCC CTG GTG GCT TTG GGA GAA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 V   D   G   D   G   D   R   K   K   R   L   A   L   V   A   L   G   E

        3250        3259        3268        3277        3286        3295
CAC GCG GAA CTA CGA AAG AGC CTT TTG CCA CCC CTC ATC ATC CAT ACG GCT GCG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 H   A   E   L   R   K   S   L   L   P   P   L   I   I   H   T   A   A

        3304        3313        3322        3331        3340        3349
ACA CCA ATG TCA CTA CCC AAG AGC TCC AGC ACA GGT GTG GGG GAA GCA CTG GGC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 T   P   M   S   L   P   K   S   S   S   T   G   V   G   E   A   L   G

        3358        3367        3376        3385        3394        3403
TCT GGC TCT CGA CGT ACC AGT AGC AGT GGG TCC GCT GAG CCT GGA GCT GCC CAC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 S   G   S   R   R   T   S   S   S   G   S   A   E   P   G   A   A   H

        3412        3421        3430        3439        3448        3457
CAT GAG ATG AAA TCT CCG CCA AGT GCC CGC AGC TCC CCG CAC AGT CCC TGG AGT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 H   E   M   K   S   P   P   S   A   R   S   S   P   H   S   P   W   S
```

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DNASIS DNA Translation [T-INS]

```
        3466        3475        3484        3493        3502        3511
GCG GCA AGC AGC TGG ACC AGC AGG CGC TCC AGC AGG AAC AGC CTG GGC CGG GCC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 A   A   S   S   W   T   S   R   R   S   S   R   N   S   L   G   R   A

        3520        3529        3538        3547        3556        3565
CCC AGC CTA AAG CGG AGG AGC CCG AGC GGG GAG CGG AGG TCC CTG CTG TCT GGA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 P   S   L   K   R   R   S   P   S   G   E   R   R   S   L   L   S   G

        3574        3583        3592        3601        3610        3619
GAG GGC CAG GAG AGT CAG GAT GAG GAG GAA AGT TCA GAA GAG GAC CGG GCC AGC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 E   G   Q   E   S   Q   D   E   E   E   S   S   E   E   D   R   A   S

        3628        3637        3646        3655        3664        3673
CCA GCA GGC AGT GAC CAT CGC CAC AGG GGT TCC TTG GAA CGT GAG GCC AAG AGT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 P   A   G   S   D   H   R   H   R   G   S   L   E   R   E   A   K   S

        3682        3691        3700        3709        3718        3727
TCC TTT GAC CTG CCT GAC ACT CTG CAG GTG CCG GGG CTG CAC CGC ACA GCC AGC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 S   F   D   L   P   D   T   L   Q   V   P   G   L   H   R   T   A   S

        3736        3745        3754        3763        3772        3781
GGC CGG AGC TCT GCC TCT GAG CAC CAA GAC TGT AAT GGC AAG TCG GCT TCA GGG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 G   R   S   S   A   S   E   H   Q   D   C   N   G   K   S   A   S   G

        3790        3799        3808        3817        3826        3835
CGT TTG GCC CGC ACC CTG AGG ACT GAT GAC CCC CAA CTG GAT GGG GAT GAT GAC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 R   L   A   R   T   L   R   T   D   D   P   Q   L   D   G   D   D   D

        3844        3853        3862        3871        3880        3889
AAT GAT GAG GGA AAT CTG AGC AAA GGG GAA CGC ATA CAA GCC TGG GTC AGA TCC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 N   D   E   G   N   L   S   K   G   E   R   I   Q   A   W   V   R   S

        3898        3907        3916        3925        3934        3943
CGG CTT CCT GCC TGT TGC CGA GAG CGA GAT TCC TGG TCG GCC TAT ATC TTT CCT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 R   L   P   A   C   C   R   E   R   D   S   W   S   A   Y   I   F   P

        3952        3961        3970        3979        3988        3997
CCT CAG TCA AGG TTT CGT CTC CTG TGT CAC CGG ATC ATC ACC CAC AAG ATG TTT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 P   Q   S   R   F   R   L   L   C   H   R   I   I   T   H   K   M   F

        4006        4015        4024        4033        4042        4051
GAC CAT GTG GTC CTC GTC ATC ATC TTC CTC AAC TGT ATC ACC ATC GCT ATG GAG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 D   H   V   V   L   V   I   I   F   L   N   C   I   T   I   A   M   E

        4060        4069        4078        4087        4096        4105
CGC CCC AAA ATT GAC CCC CAC AGC GCT GAG CGC ATC TTC CTG ACC CTC TCC AAC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 R   P   K   I   D   P   H   S   A   E   R   I   F   L   T   L   S   N

        4114        4123        4132        4141        4150        4159
TAC ATC TTC ACG GCA GTC TTT CTA GCT GAA ATG ACA GTG AAG GTG GTG GCA CTG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 Y   I   F   T   A   V   F   L   A   E   M   T   V   K   V   V   A   L
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DNASIS DNA Translation [T-INS]

```
        4168        4177        4186        4195        4204        4213
GGC TGG TGC TTT GGG GAG CAG GCC TAC CTG CGC AGC AGC TGG AAT GTG CTG GAC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 G   W   C   F   G   E   Q   A   Y   L   R   S   S   W   N   V   L   D

        4222        4231        4240        4249        4258        4267
GGC TTG CTG GTG CTC ATC TCC GTC ATC GAC ATC CTG GTC TCC ATG GTC TCC GAC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 G   L   L   V   L   I   S   V   I   D   I   L   V   S   M   V   S   D

        4276        4285        4294        4303        4312        4321
AGC GGC ACC AAG ATC CTT GGC ATG CTG AGG GTG CTG CGG CTG CTG CGG ACC CTG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 S   G   T   K   I   L   G   M   L   R   V   L   R   L   L   R   T   L

        4330        4339        4348        4357        4366        4375
CGT CCA CTC AGG GTC ATC AGC CGG GCC CAG GGA CTG AAG CTG GTG GTA GAG ACT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 R   P   L   R   V   I   S   R   A   Q   G   L   K   L   V   V   E   T

        4384        4393        4402        4411        4420        4429
CTG ATG TCA TCC CTC AAA CCC ATT GGC AAC ATT GTG GTC ATT TGC TGT GCC TTC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 L   M   S   S   L   K   P   I   G   N   I   V   V   I   C   C   A   F

        4438        4447        4456        4465        4474        4483
TTC ATC ATT TTT GGA ATT CTC GGG GTG CAG CTC TTC AAA GGG AAG TTC TTC GTG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 F   I   I   F   G   I   L   G   V   Q   L   F   K   G   K   F   F   V

        4492        4501        4510        4519        4528        4537
TGT CAG GGT GAG GAC ACC AGG AAC ATC ACT AAC AAA TCC GAC TGC GCT GAG GCC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 C   Q   G   E   D   T   R   N   I   T   N   K   S   D   C   A   E   A

        4546        4555        4564        4573        4582        4591
AGC TAC CGA TGG GTC CGG CAC AAG TAC AAC TTT GAC AAC CTG GGC CAG GCT CTG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 S   Y   R   W   V   R   H   K   Y   N   F   D   N   L   G   Q   A   L

        4600        4609        4618        4627        4636        4645
ATG TCC CTG TTT GTG CTG GCC TCC AAG GAT GGT TGG GTT GAC ATC ATG TAT GAT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 M   S   L   F   V   L   A   S   K   D   G   W   V   D   I   M   Y   D

        4654        4663        4672        4681        4690        4699
GGG CTG GAT GCT GTG GGT GTG GAT CAG CAG CCC ATC ATG AAC CAC AAC CCC TGG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 G   L   D   A   V   G   V   D   Q   Q   P   I   M   N   H   N   P   W

        4708        4717        4726        4735        4744        4753
ATG CTG CTA TAC TTC ATC TCC TTC CTC CTC ATC GTG GCC TTC TTT GTC CTG AAC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 M   L   L   Y   F   I   S   F   L   L   I   V   A   F   F   V   L   N

        4762        4771        4780        4789        4798        4807
ATG TTT GTG GGC GTG GTG GTG GAG AAC TTC CAT AAG TGC AGA CAG CAC CAG GAG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 M   F   V   G   V   V   V   E   N   F   H   K   C   R   Q   H   Q   E

        4816        4825        4834        4843        4852        4861
GAG GAG GAG GCG AGG CGG CGT GAG GAG AAG CGA CTA CGG AGG CTG GAG AAA AAG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 E   E   E   A   R   R   R   E   E   K   R   L   R   R   L   E   K   K
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DNASIS DNA Translation [T-INS]

```
        4870        4879        4888        4897        4906        4915
AGA AGG AAT CTA ATG TTG GAC GAT GTA ATT GCT TCC GGC AGC TCA GCC AGC GCT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 R   R   N   L   M   L   D   D   V   I   A   S   G   S   S   A   S   A

        4924        4933        4942        4951        4960        4969
GCG TCA GAA GCC CAG TGC AAG CCC TAC TAC TCT GAC TAC TCG AGA TTC CGG CTC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 A   S   E   A   Q   C   K   P   Y   Y   S   D   Y   S   R   F   R   L

        4978        4987        4996        5005        5014        5023
CTT GTC CAC CAC CTG TGT ACC AGC CAC TAC CTG GAC CTC TTC ATC ACT GGT GTC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 L   V   H   H   L   C   T   S   H   Y   L   D   L   F   I   T   G   V

        5032        5041        5050        5059        5068        5077
ATC GGG CTG AAC GTG GTC ACT ATG GCC ATG GAA CAT TAC CAG CAG CCC CAG ATC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 I   G   L   N   V   V   T   M   A   M   E   H   Y   Q   Q   P   Q   I

        5086        5095        5104        5113        5122        5131
CTG GAC GAG GCT CTG AAG ATC TGC AAT TAC ATC TTT ACC GTC ATC TTT GTC TTT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 L   D   E   A   L   K   I   C   N   Y   I   F   T   V   I   F   V   F

        5140        5149        5158        5167        5176        5185
GAG TCA GTT TTC AAA CTT GTG GCC TTT GGC TTC CGC CGT TTC TTC CAG GAC AGG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 E   S   V   F   K   L   V   A   F   G   F   R   R   F   F   Q   D   R

        5194        5203        5212        .5221       5230        5239
TGG AAC CAG CTG GAC CTG GCT ATT GTG CTT CTG TCC ATC ATG GGC ATC ACA CTG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 W   N   Q   L   D   L   A   I   V   L   L   S   I   M   G   I   T   L

        5248        5257        5266        5275        5284        5293
GAG GAG ATT GAG GTC AAT GCT TCG CTG CCC ATC AAC CCC ACC ATC ATC CGT ATC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 E   E   I   E   V   N   A   S   L   P   I   N   P   T   I   I   R   I

        5302        5311        5320        5329        5338        5347
ATG AGG GTG CTC CGC ATT GCT CGA GTT CTG AAG CTG TTG AAG ATG GCT GTG GGC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 M   R   V   L   R   I   A   R   V   L   K   L   L   K   M   A   V   G

        5356        5365        5374        5383        5392        5401
ATG CGG GCA CTG CTG GAC ACG GTG ATG CAG GCC CTG CCC CAG GTG GGG AAC CTG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 M   R   A   L   L   D   T   V   M   Q   A   L   P   Q   V   G   N   L

        5410        5419        5428        5437        5446        5455
GGA CTT CTC TTC ATG TTA TTG TTT TTC ATC TTT GCA GCT CTG GGC GTG GAG CTC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 G   L   L   F   M   L   L   F   F   I   F   A   A   L   G   V   E   L

        5464        5473        5482        5491        5500        5509
TTT GGA GAC CTG GAG TGT GAT GAG ACA CAC CCT TGT GAG GGC TTG GGT CGG CAT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 F   G   D   L   E   C   D   E   T   H   P   C   E   G   L   G   R   H

        5518        5527        5536        5545        5554        5563
GCC ACC TTT AGG AAC TTT GGT ATG GCC TTT CTG ACC CTC TTC CGA GTC TCC ACT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 A   T   F   R   N   F   G   M   A   F   L   T   L   F   R   V   S   T
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DNASIS DNA Translation [T-INS]

```
      5572        5581        5590        5599        5608        5617
GGT GAC AAC TGG AAT GGT ATT ATG AAG GAC ACC CTC CGG GAC TGT GAC CAG GAG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 G   D   N   W   N   G   I   M   K   D   T   L   R   D   C   D   Q   E

      5626        5635        5644        5653        5662        5671
TCC ACC TGC TAC AAC ACT GTC ATC TCC CCT ATC TAC TTT GTG TCC TTC GTG CTG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 S   T   C   Y   N   T   V   I   S   P   I   Y   F   V   S   F   V   L

      5680        5689        5698        5707        5716        5725
ACG GCC CAG TTT GTG CTG GTC AAC GTG GTC ATA GCT GTG CTG ATG AAG CAC CTG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 T   A   Q   F   V   L   V   N   V   V   I   A   V   L   M   K   H   L

      5734        5743        5752        5761        5770        5779
GAA GAA AGC AAC AAA GAG GCC AAG GAG GAG GCC GAG CTC GAG GCC GAG CTG GAG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 E   E   S   N   K   E   A   K   E   E   A   E   L   E   A   E   L   E

      5788        5797        5806        5815        5824        5833
CTG GAG ATG AAG ACG CTC AGC CCG CAG CCC CAC TCC CCG CTG GGC AGC CCC TTC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 L   E   M   K   T   L   S   P   Q   P   H   S   P   L   G   S   P   F

      5842        5851        5860        5869        5878        5887
CTC TGG CCC GGG GTG GAG GGT GTC AAC AGT CCT GAC AGC CCT AAG CCT GGG GCT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 L   W   P   G   V   E   G   V   N   S   P   D   S   P   K   P   G   A

      5896        5905        5914        5923        5932        5941
CCA CAC ACC ACT GCC CAC ATT GGA GCA GCC TCG GGC TTC TCC CTT GAG CAC CCC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 P   H   T   T   A   H   I   G   A   A   S   G   F   S   L   E   H   P

      5950        5959        5968        5977        5986        5995
ACG ATG GTA CCC CAC CCC GAG GAG GTG CCA GTC CCC CTA GGA CCA GAC CTG CTG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 T   M   V   P   H   P   E   E   V   P   V   P   L   G   P   D   L   L

      6004        6013        6022        6031        6040        6049
ACT GTG AGG AAG TCT GGT GTC AGC CGG ACG CAC TCT CTG CCC AAT GAC AGC TAC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 T   V   R   K   S   G   V   S   R   T   H   S   L   P   N   D   S   Y

      6058        6067        6076        6085        6094        6103
ATG TGC CGC AAT GGG AGC ACT GCT GAG AGA TCC CTA GGA CAC AGG GGC TGG GGG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 M   C   R   N   G   S   T   A   E   R   S   L   G   H   R   G   W   G

      6112        6121        6130        6139        6148        6157
CTC CCC AAA GCC CAG TCA GGC TCC ATC TTG TCC GTT CAC TCC CAA CCA GCA GAC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 L   P   K   A   Q   S   G   S   I   L   S   V   H   S   Q   P   A   D

      6166        6175        6184        6193        6202        6211
ACC AGC TGC ATC CTA CAG CTT CCC AAA GAT GTG CAC TAT CTG CTC CAG CCT CAT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 T   S   C   I   L   Q   L   P   K   D   V   H   Y   L   L   Q   P   H

      6220        6229        6238        6247        6256        6265
GGG GCC CCC ACC TGG GGC GCC ATC CCT AAA CTA CCC CCA CCT GGC CGC TCC CCT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 G   A   P   T   W   G   A   I   P   K   L   P   P   P   G   R   S   P
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DNASIS DNA Translation [T-INS]

```
          6274        6283        6292        6301        6310        6319
CTG GCT CAG AGG CCT CTC AGG CGC CAG GCA GCA ATA AGG ACT GAC TCC CTG GAT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 L   A   Q   R   P   L   R   R   Q   A   A   I   R   T   D   S   L   D

          6328        6337        6346        6355        6364        6373
GTG CAG GGC CTG GGT AGC CGG GAA GAC CTG TTG TCA GAG GTG AGT GGG CCC TCC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 V   Q   G   L   G   S   R   E   D   L   L   S   E   V   S   G   P   S

          6382        6391        6400        6409        6418        6427
TGC CCT CTG ACC CGG TCC TCA TCC TTC TGG GGC GGG TCG AGC ATC CAG GTG CAG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 C   P   L   T   R   S   S   S   F   W   G   G   S   S   I   Q   V   Q

          6436        6445        6454        6463        6472        6481
CAG CGT TCC GGC ATC CAG AGC AAA GTC TCC AAG CAC ATC CGC CTG CCA GCC CCT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 Q   R   S   G   I   Q   S   K   V   S   K   H   I   R   L   P   A   P

          6490        6499        6508        6517        6526        6535
TGC CCA GGC CTG GAA CCC AGC TGG GCC AAG GAC CCT CCA GAG ACC AGA AGC AGC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 C   P   G   L   E   P   S   W   A   K   D   P   P   E   T   R   S   S

          6544        6553        6562        6571        6580        6589
TTA GAG CTG GAC ACG GAG CTG AGC TGG ATT TCA GGA GAC CTC CTT CCC AGC AGC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 L   E   L   D   T   E   L   S   W   I   S   G   D   L   L   P   S   S

          6598        6607        6616        6625        6634        6643
CAG GAA GAA CCC CTG TCC CCA CGG GAC CTG AAG AAG TGC TAC AGT GTA GAG ACC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 Q   E   E   P   L   S   P   R   D   L   K   K   C   Y   S   V   E   T

          6652        6661        6670        6679        6688        6697
CAG AGC TGC AGG CGC AGG CCT GGG TCC TGG CTA GAT GAA CAG CGG AGA CAC TCC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 Q   S   C   R   R   R   P   G   S   W   L   D   E   Q   R   R   H   S

          6706        6715        6724        6733        6742        6751
ATT GCT GTC AGC TGT CTG GAC AGC GGC TCC CAA CCC CGC CTA TGT CCA AGC CCC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 I   A   V   S   C   L   D   S   G   S   Q   P   R   L   C   P   S   P

          6760        6769        6778        6787        6796        6805
TCA AGC CTC GGG GGC CAA CCT CTT GGG GGT CCT GGG AGC CGG CCT AAG AAA AAA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 S   S   L   G   G   Q   P   L   G   G   P   G   S   R   P   K   K   K

          6814        6823        6832        6841        6850        6859
CTC AGC CCA CCC AGT ATC TCT ATA GAC CCC CCG GAG AGC CAG GGC TCT CGG CCC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 L   S   P   P   S   I   S   I   D   P   P   E   S   Q   G   S   R   P

          6868        6877        6886        6895        6904        6913
CCA TGC AGT CCT GGT GTC TGC CTC AGG AGG AGG GCG CCG GCC AGT GAC TCT AAG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 P   C   S   P   G   V   C   L   R   R   R   A   P   A   S   D   S   K

          6922        6931        6940        6949        6958        6967
GAT CCC TCG GTC TCC AGC CCC CTT GAC AGC ACG GCT GCC TCA CCC TCC CCA AAG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 D   P   S   V   S   S   P   L   D   S   T   A   A   S   P   S   P   K
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DNASIS DNA Translation [T-INS]

```
        6976        6985        6994        7003        7012        7021
AAA GAC ACG CTG AGT CTC TCT GGT TTG TCT TCT GAC CCA ACA GAC ATG GAC CCC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 K   D   T   L   S   L   S   G   L   S   S   D   P   T   D   M   D   P

        7030        7039        7048        7057        7066        7075
TGA GTC CTA CCC ACT CTC CCC CAT CAC CTT TCT CCA CCG GGT GCA GAT CCT AGC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 *   V   L   P   T   L   P   H   H   L   S   P   P   G   A   D   P   S

        7084        7093        7102        7111        7120        7129
TCC GCC TCC TGG GCA GCG TTT CTG AAA AGT CCC ACG TAA GCA GCA AGC AGC CAC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 S   A   S   W   A   A   F   L   K   S   P   T   *   A   A   S   S   H

        7138        7147        7156        7165        7174        7183
GAG GCA CCT CAC CTG CCT TCT TCA GTG GCT GGT GGG GAT GAC GAG CAG AAC TTC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 E   A   P   H   L   P   S   S   V   A   G   G   D   D   E   Q   N   F

        7192        7201        7210        7219        7228        7237
CGG AGA GTC GAT CTG AAG AGA ACA CAG CCC TGG AGC CCC TGC CTC CGG GAA GAA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 R   R   V   D   L   K   R   T   Q   P   W   S   P   C   L   R   E   E

        7246        7255        7264        7273        7282
GGA AAA GGA GAA AGC CCA GTG TGG CCA AGG CTC CCG ACA CCA GGA GCT G/3'
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 G   K   G   E   S   P   V   W   P   R   L   P   T   P   G   A/
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end of SEQ 1 + 3

end of SEQ 2 + 4

DNASIS
T-INS

SEQ ID NO:3

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        10          20          30          40          50          60
GGAGCTGAGC TGAACTGGCC CTCCTGGGGA CTCAGCAAGC TCTCTAGAGC CCCCCACATG

        70          80          90         100         110         120
CTCCCCCACC GGGGTCCCCC GGTTGCGTGA GGACACCTCC TCTGAGGGGC TCCGCTCGCC

       130         140         150         160         170         180
CCTCTTCGGA CCCCCCGGGG CCCCGGCTGG CCAGAGGATG GACGAGGAGG AGGATGGAGC

       190         200         210         220         230         240
GGGCGCCGAG GAGTCGGGAC AGCCCCGTAG CTTCACGCAG CTCAACGACC TGTCCGGGGC

       250         260         270         280         290         300
CGGGGGCCGG CAGGGGCCGG GGTCGACGGA AAAGGACCCG GGCAGCGCGG ACTCCGAGGC

       310         320         330         340         350         360
GGAGGGGCTG CCGTACCCGG CGCTAGCCCC GGTGGTTTTC TTCTACTTGA GCCAGGACAG

       370         380         390         400         410         420
CCGCCCGCGG AGCTGGTGTC TCCGCACGGT CTGTAACCCG TGGTTCGAGC GAGTCAGTAT

       430         440         450         460         470         480
GCTGGTCATT CTTCTCAACT GTGTGACTCT GGGTATGTTC AGGCCGTGTG AGGACATTGC

       490         500         510         520         530         540
CTGTGACTCC CAGCGCTGCC GGATCCTGCA GGCCTTCGAT GACTTCATCT TTGCCTTCTT

       550         560         570         580         590         600
TGCTGTGGAA ATGGTGGTGA AGATGGTGGC CTTGGGCATC TTTGGGAAGA AATGTTACCT

       610         620         630         640         650         660
GGGAGACACT TGGAACCGGC TTGACTTTTT CATTGTCATT GCAGGGATGC TGGAGTATTC

       670         680         690         700         710         720
GCTGGACCTG CAGAACGTCA GCTTCTCCGC AGTCAGGACA GTCCGTGTGC TGCGACCGCT

       730         740         750         760         770         780
CAGGGCCATT AACCGGGTGC CCAGCATGCG CATTCTCGTC ACATTACTGC TGGACACCTT

       790         800         810         820         830         840
GCCTATGCTG GGCAACGTCC TGCTGCTCTG TTTCTTCGTC TTTTTCATCT TTGGCATCGT

       850         860         870         880         890         900
GGGCGTCCAG CTGTGGGCAG GACTGCTTCG CAACCGATGC TTCCTCCCCG AGAACTTCAG

       910         920         930         940         950         960
CCTCCCCCTG AGCGTGGACC TGGAGCCTTA TTACCAGACA GAGAATGAGG ACGAGAGCCC

       970         980         990        1000        1010        1020
CTTCATCTGC TCTCAGCCTC GGGAGAATGG CATGAGATCC TGCAGGAGTG TGCCCACACT

      1030        1040        1050        1060        1070        1080
GCGTGGGGAA GGCGGTGGTG GCCCACCCTG CAGTCTGGAC TATGAGACCT ATAACAGTTC

      1090        1100        1110        1120        1130        1140
CAGCAACACC ACCTGTGTCA ACTGGAACCA GTACTATACC AACTGCTCTG CGGGCGAGCA

      1150        1160        1170        1180        1190        1200
CAACCCTTC AAAGGCGCCA TCAACTTTGA CAACATTGGC TATGCCTGGA TCGCCATCTT

      1210        1220        1230        1240        1250        1260
CCAGGTCATC ACACTGGAGG GCTGGGTCGA CATCATGTAC TTCGTAATGG ACGCTCACTC
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start of SEQ ID NO:1 coding region

DNASIS
T-INS

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      1270        1280        1290        1300        1310        1320
CTTCTACAAC TTCATCTACT TCATTCTTCT CATCATCGTG GGCTCCTTCT TCATGATCAA

      1330        1340        1350        1360        1370        1380
CCTGTGCCTG GTGGTGATTG CCACGCAGTT CTCCGAGACC AAACAGCGGG AGAGTCAGCT

      1390        1400        1410        1420        1430        1440
GATGCGGGAG CAGCGTGTAC GATTCCTGTC CAATGCTAGC ACCCTGGCAA GCTTCTCTGA

      1450        1460        1470        1480        1490        1500
GCCAGGCAGC TGCTATGAGG AGCTACTCAA GTACCTGGTG TACATCCTCC GAAAAGCAGC

      1510        1520        1530        1540        1550        1560
CCGAAGGCTG GCCCAGGTCT CTAGGGCTAT AGGCGTGCGG GCTGGGCTGC TCAGCAGCCC

      1570        1580        1590        1600        1610        1620
AGTGGCCCGT AGTGGGCAGG AGCCCCAGCC CAGTGGCAGC TGCACTCGCT CACACCGTCG

      1630        1640        1650        1660        1670        1680
TCTGTCTGTC CACCACCTGG TCCACCACCA TCACCACCAC CATCACCACT ACCACCTGGG

      1690        1700        1710        1720        1730        1740
TAATGGGACG CTCAGAGTTC CCCGGGCCAG CCCAGAGATC CAGGACAGGG ATGCCAATGG

      1750        1760        1770        1780        1790        1800
GTCTCGCCGG CTCATGCTAC CACCACCCTC TACACCCACT CCCTCTGGGG GCCCTCCGAG

      1810        1820        1830        1840        1850        1860
GGGTGCGGAG TCTGTACACA GCTTCTACCA TGCTGACTGC CACTTGGAGC CAGTCCGTTG

      1870        1880        1890        1900        1910        1920
CCAGGCACCC CCTCCCAGAT GCCCATCGGA GGCATCTGGT AGGACTGTGG GTAGTGGGAA

      1930        1940        1950        1960        1970        1980
GGTGTACCCC ACTGTGCATA CCAGCCCTCC ACCAGAGATA CTGAAGGATA AAGCACTAGT

      1990        2000        2010        2020        2030        2040
GGAGGTGGCC CCCAGCCCTG GGCCCCCCAC CCTCACCAGC TTCAACATCC CACCTGGGCC

      2050        2060        2070        2080        2090        2100
CTTCAGCTCC ATGCACAAGC TCCTGGAGAC ACAGAGTACG GGAGCCTGCC ATAGCTCCTG

      2110        2120        2130        2140        2150        2160
CAAAATCTCC AGCCCTTGCT CCAAGGCAGA CAGTGGAGCC TGCGGGCCGG ACAGTTGTCC

      2170        2180        2190        2200        2210        2220
CTACTGTGCC CGGACAGGAG CAGGAGAGCC AGAGTCCGCT GACCATGTCA TGCCTGACTC

      2230        2240        2250        2260        2270        2280
AGACAGCGAG GCTGTGTATG AGTTCACACA GGACGCTCAG CACAGTGACC TCCGGGATCC

      2290        2300        2310        2320        2330        2340
CCACAGCCGG CGGCGACAGC GGAGCCTGGG CCCAGATGCA GAGCCTAGTT CTGTGCTGGC

      2350        2360        2370        2380        2390        2400
TTTCTGGAGG CTGATCTGTG ACACATTCCG GAAGATCGTA GATAGCAAAT ACTTTGGCCG

      2410        2420        2430        2440        2450        2460
GGGAATCATG ATCGCCATCC TGGTCAATAC ACTCAGCATG GGCATCGAGT ACCACGAGCA

      2470        2480        2490        2500        2510        2520
GCCCGAGGAG CTCACCAACG CCCTGGAAAT CAGCAACATC GTCTTCACCA GCCTCTTCGC
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DNASIS
T-INS

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      2530        2540        2550        2560        2570        2580
CTTGGAGATG CTGCTGAAAC TGCTTGTCTA CGGTCCCTTT GGCTACATTA AGAATCCCTA

      2590        2600        2610        2620        2630        2640
CAACATCTTT GATGGTGTCA TTGTGGTCAT CAGTGTGTGG GAGATTGTGG GCCAGCAGGG

      2650        2660        2670        2680        2690        2700
AGGTGGCCTG TCGGTGCTGC GGACCTTCCG CCTGATGCGG GTGCTGAAGC TGGTGCGCTT

      2710        2720        2730        2740        2750        2760
CCTGCCGGCC CTGCAGCGCC AGCTCGTGGT GCTCATGAAG ACCATGGACA ACGTGGCCAC

      2770        2780        2790        2800        2810        2820
CTTCTGCATG CTCCTCATGC TGTTCATCTT CATCTTCAGC ATCCTGGGCA TGCATCTCTT

      2830        2840        2850        2860        2870        2880
TGGTTGCAAG TTCGCATCTG AACGGGATGG GGACACGTTG CCAGACCGGA AGAATTTCGA

      2890        2900        2910        2920        2930        2940
CTCCCTGCTC TGGGCCATCG TCACTGTCTT TCAGATTCTG ACTCAGGAAG ACTGGAATAA

      2950        2960        2970        2980        2990        3000
AGTCCTCTAC AACGGCATGG CCTCCACATC GTCTTGGGCT GCTCTTTACT TCATCGCCCT

      3010        3020        3030        3040        3050        3060
CATGACTTTT GGCAACTATG TGCTCTTTAA CCTGCTGGTG GCCATTCTTG TGGAAGGATT

      3070        3080        3090        3100        3110        3120
CCAGGCAGAG GAAATCGGCA AACGGGAAGA TGCGAGTGGA CAGTTAAGCT GTATTCAGCT

      3130        3140        3150        3160        3170        3180
GCCTGTCAAC TCTCAGGGGG GAGATGCCAC CAAGTCTGAG TCAGAGCCTG ATTTCTTTTC

      3190        3200        3210        3220        3230        3240
GCCCAGTGTG GATGGTGATG GGGACAGAAA GAAGCGCTTG GCCCTGGTGG CTTTGGGAGA

      3250        3260        3270        3280        3290        3300
ACACGCGGAA CTACGAAAGA GCCTTTTGCC ACCCCTCATC ATCCATACGG CTGCGACACC

      3310        3320        3330        3340        3350        3360
AATGTCACTA CCCAAGAGCT CCAGCACAGG TGTGGGGGAA GCACTGGGCT CTGGCTCTCG

      3370        3380        3390        3400        3410        3420
ACGTACCAGT AGCAGTGGGT CCGCTGAGCC TGGAGCTGCC CACCATGAGA TGAAATCTCC

      3430        3440        3450        3460        3470        3480
GCCAAGTGCC CGCAGCTCCC CGCACAGTCC CTGGAGTGCG GCAAGCAGCT GGACCAGCAG

      3490        3500        3510        3520        3530        3540
GCGCTCCAGC AGGAACAGCC TGGGCCGGGC CCCCAGCCTA AAGCGGAGGA GCCCGAGCGG

      3550        3560        3570        3580        3590        3600
GGAGCGGAGG TCCCTGCTGT CTGGAGAGGG CCAGGAGAGT CAGGATGAGG AGGAAAGTTC

      3610        3620        3630        3640        3650        3660
AGAAGAGGAC CGGGCCAGCC CAGCAGGCAG TGACCATCGC CACAGGGGTT CCTTGGAACG

      3670        3680        3690        3700        3710        3720
TGAGGCCAAG AGTTCCTTTG ACCTGCCTGA CACTCTGCAG GTGCCGGGGC TGCACCGCAC

      3730        3740        3750        3760        3770        3780
AGCCAGCGGC CGGAGCTCTG CCTCTGAGCA CCAAGACTGT AATGGCAAGT CGGCTTCAGG
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DNASIS
T-INS

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      3790        3800        3810        3820        3830        3840
GCGTTTGGCC CGCACCCTGA GGACTGATGA CCCCCAACTG GATGGGGATG ATGACAATGA

      3850        3860        3870        3880        3890        3900
TGAGGGAAAT CTGAGCAAAG GGGAACGCAT ACAAGCCTGG GTCAGATCCC GGCTTCCTGC

      3910        3920        3930        3940        3950        3960
CTGTTGCCGA GAGCGAGATT CCTGGTCGGC CTATATCTTT CCTCCTCAGT CAAGGTTTCG

      3970        3980        3990        4000        4010        4020
TCTCCTGTGT CACCGGATCA TCACCCACAA GATGTTTGAC CATGTGGTCC TCGTCATCAT

      4030        4040        4050        4060        4070        4080
CTTCCTCAAC TGTATCACCA TCGCTATGGA GCGCCCCAAA ATTGACCCCC ACAGCGCTGA

      4090        4100        4110        4120        4130        4140
GCGCATCTTC CTGACCCTCT CCAACTACAT CTTCACGGCA GTCTTTCTAG CTGAAATGAC

      4150        4160        4170        4180        4190        4200
AGTGAAGGTG GTGGCACTGG GCTGGTGCTT TGGGGAGCAG GCCTACCTGC GCAGCAGCTG

      4210        4220        4230        4240        4250        4260
GAATGTGCTG GACGGCTTGC TGGTGCTCAT CTCCGTCATC GACATCCTGG TCTCCATGGT

      4270        4280        4290        4300        4310        4320
CTCCGACAGC GGCACCAAGA TCCTTGGCAT GCTGAGGGTG CTGCGGCTGC TGCGGACCCT

      4330        4340        4350        4360        4370        4380
GCGTCCACTC AGGGTCATCA GCCGGGCCCA GGGACTGAAG CTGGTGGTAG AGACTCTGAT

      4390        4400        4410        4420        4430        4440
GTCATCCCTC AAACCCATTG GCAACATTGT GGTCATTTGC TGTGCCTTCT TCATCATTTT

      4450        4460        4470        4480        4490        4500
TGGAATTCTC GGGGTGCAGC TCTTCAAAGG GAAGTTCTTC GTGTGTCAGG GTGAGGACAC

      4510        4520        4530        4540        4550        4560
CAGGAACATC ACTAACAAAT CCGACTGCGC TGAGGCCAGC TACCGATGGG TCCGGCACAA

      4570        4580        4590        4600        4610        4620
GTACAACTTT GACAACCTGG GCCAGGCTCT GATGTCCCTG TTTGTGCTGG CCTCCAAGGA

      4630        4640        4650        4660        4670        4680
TGGTTGGGTT GACATCATGT ATGATGGGCT GGATGCTGTG GGTGTGGATC AGCAGCCCAT

      4690        4700        4710        4720        4730        4740
CATGAACCAC AACCCCTGGA TGCTGCTATA CTTCATCTCC TTCCTCCTCA TCGTGGCCTT

      4750        4760        4770        4780        4790        4800
CTTTGTCCTG AACATGTTTG TGGGCGTGGT GGTGGAGAAC TTCCATAAGT GCAGACAGCA

      4810        4820        4830        4840        4850        4860
CCAGGAGGAG GAGGAGGCGA GGCGGCGTGA GGAGAAGCGA CTACGGAGGC TGGAGAAAAA

      4870        4880        4890        4900        4910        4920
GAGAAGGAAT CTAATGTTGG ACGATGTAAT TGCTTCCGGC AGCTCAGCCA GCGCTGCGTC

      4930        4940        4950        4960        4970        4980
AGAAGCCCAG TGCAAGCCCT ACTACTCTGA CTACTCGAGA TTCCGGCTCC TTGTCCACCA

      4990        5000        5010        5020        5030        5040
CCTGTGTACC AGCCACTACC TGGACCTCTT CATCACTGGT GTCATCGGGC TGAACGTGGT
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DNASIS
T-INS

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      5050        5060        5070        5080        5090        5100
CACTATGGCC ATGGAACATT ACCAGCAGCC CCAGATCCTG GACGAGGCTC TGAAGATCTG

      5110        5120        5130        5140        5150        5160
CAATTACATC TTTACCGTCA TCTTTGTCTT TGAGTCAGTT TTCAAACTTG TGGCCTTTGG

      5170        5180        5190        5200        5210        5220
CTTCCGCCGT TTCTTCCAGG ACAGGTGGAA CCAGCTGGAC CTGGCTATTG TGCTTCTGTC

      5230        5240        5250        5260        5270        5280
CATCATGGGC ATCACACTGG AGGAGATTGA GGTCAATGCT TCGCTGCCCA TCAACCCCAC

      5290        5300        5310        5320        5330        5340
CATCATCCGT ATCATGAGGG TGCTCCGCAT TGCTCGAGTT CTGAAGCTGT TGAAGATGGC

      5350        5360        5370        5380        5390        5400
TGTGGGCATG CGGGCACTGC TGGACACGGT GATGCAGGCC CTGCCCCAGG TGGGGAACCT

      5410        5420        5430        5440        5450        5460
GGGACTTCTC TTCATGTTAT TGTTTTTCAT CTTTGCAGCT CTGGGCGTGG AGCTCTTTGG

      5470        5480        5490        5500        5510        5520
AGACCTGGAG TGTGATGAGA CACACCCTTG TGAGGGCTTG GGTCGGCATG CCACCTTTAG

      5530        5540        5550        5560        5570        5580
GAACTTTGGT ATGGCCTTTC TGACCCICTT CCGAGTCTCC ACTGGTGACA ACTGGAATGG

      5590        5600        5610        5620        5630        5640
TATTATGAAG GACACCCTCC GGGACTCTGA CCAGGAGTCC ACCTGCTACA ACACTGTCAT

      5650        5660        5670        5680        5690        5700
CTCCCCTATC TACTTTGTGT CCTTCGTGCT GACGGCCCAG TTTGTGCTGG TCAACGTGGT

      5710        5720        5730        5740        5750        5760
CATAGCTGTG CTGATGAAGC ACCTGGAAGA AAGCAACAAA GAGGCCAAGG AGGAGGCCGA

      5770        5780        5790        5800        5810        5820
GCTCGAGGCC GAGCTGGAGC TGGAGATGAA GACGCTCAGC CCGCAGCCCC ACTCCCCGCT

      5830        5840        5850        5860        5870        5880
GGGCAGCCCC TTCCTCTGGC CCGGGGTGGA GGGTGTCAAC AGTCCTGACA GCCCTAAGCC

      5890        5900        5910        5920        5930        5940
TGGGGCTCCA CACACCACTG CCCACATTGG AGCAGCCTCG GGCTTCTCCC TTGAGCACCC

      5950        5960        5970        5980        5990        6000
CACGATGGTA CCCCACCCCG AGGAGGTGCC AGTCCCCCTA GGACCAGACC TGCTGACTGT

      6010        6020        6030        6040        6050        6060
GAGGAAGTCT GGTGTCAGCC GGACGCACTC TCTGCCCAAT GACAGCTACA TGTGCCGCAA

      6070        6080        6090        6100        6110        6120
TGGGAGCACT GCTGAGAGAT CCCTAGGACA CAGGGGCTGG GGGCTCCCCA AAGCCCAGTC

      6130        6140        6150        6160        6170        6180
AGGCTCCATC TTGTCCGTTC ACTCCCAACC AGCAGACACC AGCTGCATCC TACAGCTTCC

      6190        6200        6210        6220        6230        6240
CAAAGATGTG CACTATCTGC TCCAGCCTCA TGGGGCCCCC ACCTGGGGCG CCATCCCTAA

      6250        6260        6270        6280        6290        6300
ACTACCCCCA CCTGGCCGCT CCCCTCTGGC TCAGAGGCCT CTCAGGCGCC AGGCAGCAAT
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DNASIS
T-INS

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AAGGACTGAC TCCCTGGATG TGCAGGGCCT GGGTAGCCGG GAAGACCTGT TGTCAGAGGT

      6370        6380        6390        6400        6410        6420
GAGTGGGCCC TCCTGCCCTC TGACCCGGTC CTCATCCTTC TGGGGCGGGT CGAGCATCCA

      6430        6440        6450        6460        6470        6480
GGTGCAGCAG CGTTCCGGCA TCCAGAGCAA AGTCTCCAAG CACATCCGCC TGCCAGCCCC

      6490        6500        6510        6520        6530        6540
TTGCCCAGGC CTGGAACCCA GCTGGGCCAA GGACCCTCCA GAGACCAGAA GCAGCTTAGA

      6550        6560        6570        6580        6590        6600
GCTGGACACG GAGCTGAGCT GGATTTCAGG AGACCTCCTT CCCAGCAGCC AGGAAGAACC

      6610        6620        6630        6640        6650        6660
CCTGTCCCCA CGGGACCTGA AGAAGTGCTA CAGTGTAGAG ACCCAGAGCT GCAGGCGCAG

      6670        6680        6690        6700        6710        6720
GCCTGGGTCC TGGCTAGATG AACAGCGGAG ACACTCCATT GCTGTCAGCT GTCTGGACAG

      6730        6740        6750        6760        6770        6780
CGGCTCCCAA CCCCGCCTAT GTCCAAGCCC CTCAAGCCTC GGGGGCCAAC CTCTTGGGGG

      6790        6800        6810        6820        6830        6840
TCCTGGGAGC CGGCCTAAGA AAAAACTCAG CCCACCCAGT ATCTCTATAG ACCCCCCGGA

      6850        6860        6870        6880        6890        6900
GAGCCAGGGC TCTCGGCCCC CATGCAGTCC TGGTGTCTGC CTCAGGAGGA GGGCGCCGGC

      6910        6920        6930        6940        6950        6960
CAGTGACTCT AAGGATCCCT CGGTCTCCAG CCCCCTTGAC AGCACGGCTG CCTCACCCTC

      6970        6980        6990        7000        7010        7020
CCCAAAGAAA GACACGCTGA GTCTCTCTGG TTTGTCTTCT GACCCAACAG ACATGGACCC

      7030        7040        7050        7060        7070        7080
CTGAGTCCTA CCCACTCTCC CCCATCACCT TTCTCCACCG GGTGCAGATC CTAGCTCCGC

      7090        7100        7110        7120        7130        7140
CTCCTGGGCA GCGTTTCTGA AAAGTCCCAC GTAAGCAGCA AGCAGCCACG AGGCACCTCA

      7150        7160        7170        7180        7190        7200
CCTGCCTTCT TCAGTGGCTG GTGGGGATGA CGAGCAGAAC TTCCGGAGAG TCGATCTGAA

      7210        7220        7230        7240        7250        7260
GAGAACACAG CCCTGGAGCC CCTGCCTCCG GGAAGAAGGA AAAGGAGAAA GCCCAGTGTG

      7270        7280        7290        7300        7310        7320
GCCAAGGCTC CCGACACCAG GAGCTG/... .......... .......... ..........
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End of SEQ 1+3

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SEQ ID NO:4

start of SEQ ID NO:2 (coding region)

ELS*TGPPGDSASSLEPPTCSPTGVPRLREDTSSEGLRSPLFGPPG
APAGQR[redacted]DEEEDGAGAEESGQPRSFTQLNDLSGAGGRQGPGSTEK
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RVSMLVILLNCVTLGMFRPCEDIACDSQRCRILQAFDDFIFAFFAV
EMVVKMVALGIFGKKCYLGDTWNRLDFFIVIAGMLEYSLDLQNVS
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KTMDNVATFCMLLMLFIFIFSILGMHLFGCKFASERDGDTLPDRK
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FGNYVLFNLLVAILVEGFQAEEIGKREDASGQLSCIQLPVNSQGGD
ATKSESEPDFFSPSVDGDGDRKKRLALVALGEHAELRKSLLPPLII
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SDCAEASYRWVRHKYNFDNLGQALMSLFVLASKDGWVDIMYDGL
DAVGVDQQPIMNHNPWMLLYFISFLLIVAFFVLNMFVGVVVENFH
KCRQHQEEEEARRREEKRLRRLEKKRRNLMLDDVIASGSSASAAS
EAQCKPYYSDYSRFRLLVHHLCTSHYLDLFITGVIGLNVVTMAME
HYQQPQILDEALKICNYIFTVIFVFESVFKLVAFGFRRFFQDRWNQ
LDLAIVLLSIMGITLEEIEVNASLPINPTIIRIMRVLRIARVLKLLKM
AVGMRALLDTVMQALPQVGNLGLLFMLLFFIFAALGVELFGDLEC
DETHPCEGLGRHATFRNFGMAFLTLFRVSTGDNWNGIMKDTLRDC
DQESTCYNTVISPIYFVSFVLTAQFVLVNVVIAVLMKHLEESNKEA
KEEAELEAELELEMKTLSPQPHSPLGSPFLWPGVEGVNSPDSPKPG
APHTTAHIGAASGFSLEHPTMVPHPEEVPVPLGPDLLTVRKSGVSR
THSLPNDSYMCRNGSTAERSLGHRGWGLPKAQSGSILSVHSQPAD
TSCILQLPKDVHYLLQPHGAPTWGAIPKLPPPGRSPLAQRPLRRQA
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SGIQSKVSKHIRLPAPCPGLEPSWAKDPPETRSSLELDTELSWISG
DLLPSSQEEPLSPRDLKKCYSVETQSCRRRPGSWLDEQRRHSIAV
SCLDSGSQPRLCPSPSSLGGQPLGGPGSRPKKKLSPPSISIDPPESQ
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SGLSSDPTDMDP*VLPTLPHHLSPPGADPSSASWAAFLKSPT*AAS
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SPVWPRLPTPGA/

end of SEQ 2+4

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